# THE STERILIZATION OF SWEET CREAM FOR MARKET PURPOSES*

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The preparation of a sweet, sterile cream put into small containers, to be used for retail trade, offers certain technical difficulties. It is hoped that the results reported in this paper will help to overcome these and to facilitate the production of a cream which it is believed will have a definite and useful rôle in our increasing list of dairy products.

A method of preparing sterile cream has been described in a public patent issued to the author (1) and the fundamental relations governing the heat stability of homogenized creams have been investigated by Webb and Holm (2).

Sweet cream containing 20 per cent butterfat will withstand a sterilization temperature of 120°C. for 90 to 140 minutes before coagulation. To prevent separation of fat during long periods of storage however cream must be homogenized at relatively high pressures. Such treatment invariably lowers the heat stability of the product, due to factors as yet unexplained.

## EXPERIMENTAL

To obtain a complete picture of the effect of homogenization upon the heat stability of cream containing 20 per cent butterfat, numerous samples were preheated to 60°, 70°, 80°, and 90°C. without holding, each being homogenized at pressures of from 500 to 4000 pounds as soon as the preheating temperature was attained. These samples were subsequently sterilized at 118°C. until coagulation occurred. Considerable variation in stability was found for different creams, but the relative effect upon stability of preheating before homogenization was the same. Representative data for a normal cream are plotted in figure 1.

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Variations in butterfat content were found to produce marked changes in stability after homogenization. Increases beyond 20 per cent lowered stability, whereas decreases increased it. Data showing this relationship for creams of three butterfat percentages are given in table 1. (See also Webb and Holm (2).)

Homogenization of cream containing 20 per cent butterfat at 2800 to 3200 pounds pressure will largely prevent separation of fat in storage. In the samples prepared a soft cream plug always

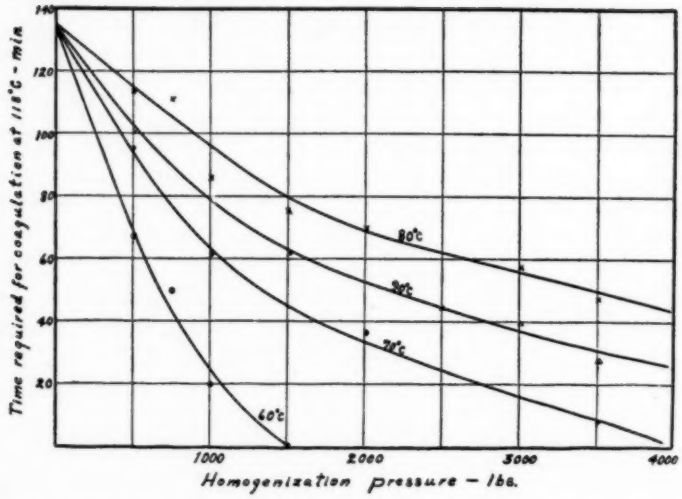


FIG. 1. EFFECT OF PREHEATING TEMPERATURE UPON THE HEAT STABILITY OF 20 PER CENT CREAM HOMOGENIZED AT VARIOUS PRESSURES

formed after a few months' undisturbed storage at room temperature. Nevertheless, this separation was not greatly increased even after two years, and the cream plug readily dissolved, especially when warm, with a little shaking. It was found that by storing the bottles upside down the cream plug more readily disintegrated upon shaking and in no way obstructed the narrow neck of the bottle.

Samples of sterile cream were scored for flavor, on the basis of 15 as perfect. This was also done for creams sterilized at differ-

ent times and temperatures and stored at room temperature for various periods up to 2½ years, when the flavor was still good and the cream would have passed as coffee cream or cream for cereal or fruit. Some of the representative data upon flavor are given

TABLE 1

*Effect of variation in butterfat content upon the heat stability of creams preheated at different temperatures*

| PREHEATING TEMPERATURE | HOMOGENIZATION PRESSURE | TIME OF COAGULATION AT 118°C. | | |
|---|---|---|---|---|
| | | 15 per cent butterfat | 20 per cent butterfat | 25 per cent butterfat |
| °C. | pounds per square inch | minutes | minutes | minutes |
| Not heated | 0 | 140 | 136 | 128 |
| 60 | 3,000 | 27 | 1 | −0 |
| 80 | 3,000 | 85 | 52 | 18 |

TABLE 2

*Effect of time and temperature of sterilization and of time of storage upon the flavor of sterilized cream**

| TEMPERATURE OF STERILIZATION | CHECK, FRESH CREAM, NO STERILIZATION | TIME OF STORAGE AT ROOM TEMPERATURE | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 hours | | | 1 month | | | 3 months | | | 2½ years | |
| | | Time of sterilization in minutes | | | | | | | | | | |
| | | 8 | 12 | 16 | 8 | 12 | 16 | 8 | 12 | 16 | 12 | |
| °C. | | | | | | | | | | | | |
| 108 | 13.5 | | 11.9 | | | 10.1 | | | 9.5 | | 6.8 | Pronounced old cream flavor |
| 112 | 13.5 | 11.7 | 11.1 | 10.7 | 10.0 | 10.1 | 10.3 | 9.9 | 9.9 | | 0.0 | Rancid |
| 115 | 13.5 | 11.3 | 10.8 | 10.2 | 10.5 | 10.5 | 10.3 | 10.1 | 10.3 | 10.3 | 8.8 | Old cream flavor |
| 118 | 13.5 | 10.8 | 10.6 | 10.4 | 10.5 | 10.5 | 10.5 | 10.1 | 10.3 | 10.4 | 9.2 | Old cream flavor |
| 122 | 13.5 | | 10.0 | | | 10.1 | | | 10.0 | | 9.2 | Old cream flavor |

* Score for flavor on basis of 15 for perfect.

in table 2. Sterilization at 118°C. for 12 to 14 minutes seemed to produce a cream with the best keeping quality and flavor.

Several types of containers for the sterile products were used. It was found that when put up in tin cans, the cream always developed an undesirable rancid-like flavor, although when pieces of

tin were placed in the glass containers the cream did not have the flavor characteristic of that in the tin containers. The use of evacuated glass jars and of evacuated tin cans proved to have no more advantage in improving the flavor of the product after storage than did the same containers sealed in air.

The container finally decided upon as the most practical and as one which did not change the flavor of the cream was a 250-cc. white-glass bottle similar to a grape-juice or soda-water bottle. The caps used were lined with parchment or special cardboard rather than composition cork, which blackened the top layer of cream with which it came in contact. These bottles were easily sealed and withstood sterilization without breakage or loss of tops.

Observations upon the color of the product were made by comparison with fixed color tubes prepared according to Webb and Holm (3) by adding varying amounts of a 10 per cent $FeCl_3$ solution to a 1:5 $CaCO_3$ suspension, to match cream colors.

There was a slight but noticeable darkening in color after sterilization and a very slight darkening up to the first month of storage, after which no significant change in color was detected.

Relative viscosity during storage was measured, but no important relationship was found between length of storage of the creams sterilized at various times and temperatures and viscosity.

An investigation of the whipping properties of sterile cream was carried out. The work of Babcock (4) on the whipping quality of cream was found of value in its application to sterile cream. Such creams were prepared with a butterfat content of from 20 to 35 per cent. When creams with a butterfat percentage above 30 were homogenized at more than 2000 pounds pressure they seldom withstood sterilization at 118°C. for 12 minutes. Heat treatment alone only slightly lowered whipping quality, but homogenization of the product seriously decreased the whipping ability of a cream, approximately in proportion to the pressure applied. Whipping temperature was found to be important, the optimum temperature being 5° to 12°C. Homogenized cream of 20 per cent butterfat would not whip. Fair whipping quality was observed in sterile creams of 25 to 35 per cent fat homoge-

nized up to 2000 pounds pressure, but the resulting product showed but little increase in volume, although a very stiff whip could be obtained. Creams having higher percentages of fat (30 to 35 per cent, homogenized at 2500 pounds) showed great separation of the fat after a year of storage, but readily mixed and their whipping properties were only slightly decreased as a result of storage.

## DISCUSSION

The preheating of a good quality cream containing 20 per cent butterfat to 80°C. before homogenization allows the product to be sterilized with no coagulation. The cooked flavor of such a cream is not excessive and when eaten with fruit or cereal or in coffee would often be unnoticed. This cooked flavor is not so pronounced as that of evaporated milk, due probably to the much smaller percentage of solids not fat. After a considerable period of storage however, a slight "milk powder" or "old cream" flavor develops.

There is no marked or objectionable change in color or viscosity of the product during preparation or storage.

It is believed that an attractively packaged, sweet, sterile, cream would have excellent market possibilities where fresh cream is not available or where it is desirable to keep on hand a good quality cream for use on various occasions.

The possibilities of preparing a sterile whipping cream are less encouraging. Cream of 20 per cent butterfat will not whip easily, due to the high pressure of homogenization necessary to prevent separation. A sterile cream of a higher percentage of fat can be prepared but it is not entirely satisfactory because of the tendency of the fat to separate and the small increase in volume observed after whipping.

Optimum conditions for the preparation of a sterile whipping cream were attained when 30 per cent cream was preheated to 80°C., homogenized at 2500 pounds pressure, and sterilized at 118°C. for 12 minutes. This cream whipped well at 10°C. but with only a small increase in volume. A noticeable separation and an old cream flavor resulted after a year of storage of this product, although its whipping ability was still retained.

SUMMARY

The important factors in the preparation of a sweet, sterile cream have been discussed. Such a product may best be prepared by preheating a cream containing 20 per cent butterfat to 80°C., homogenizing at 3000 pounds' pressure, cooling, placing in bottles of the soda water type, and sterilizing at 118°C. for 12 to 14 minutes. This product will not whip easily. It will keep indefinitely without a seriously objectionable change in flavor or separation of the butterfat.

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# THE FEED REQUIREMENTS AND THE FEED COST OF THE DAIRY SIRE*

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One of the necessary expenses of maintaining a dairy herd is the herd sire. The census of 1920 showed a total of 772,320 bulls one year and older in the United States. Apparently about one farmer out of six having dairy cattle keeps a bull. Unquestionably one reason for the widespread use of inferior sires is the considerable expense involved in the purchase and maintenance of a first class animal.

The purpose of the investigation reported was to determine: (1) the feed cost of keeping a bull under farm conditions and (2) to ascertain the amount of nutrients required to keep a bull in proper breeding condition.

The literature dealing with dairy management presents but little in the way of data concerning the feed requirements of the bull. The recommendations regarding the character of the ration are mostly in generalities. The usual recommendations are to feed sufficiently liberal to keep the sire in a vigorous condition but not too fat. Leguminous roughage is commonly recommended to be fed at the rate of 10 to 15 pounds daily with an allowance of corn silage not to exceed 15 pounds daily. The feeding of concentrates to the amount of from 4 to 8 pounds is generally suggested. A typical grain mixture selected from the various ones suggested is corn or barley 300 pounds, oats or bran 200 pounds and linseed oilmeal or other high protein concentrate 100 pounds.

Valuable data are available taken by the United States Department of Agriculture (1) on dairy farms in the states of Vermont, Indiana, Washington, and Louisiana. The records obtained were part of those taken in connection with a study of the unit

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requirements for producing market milk in the regions indicated. Each farm was visited at monthly intervals by an agent who obtained the data required. For obvious reasons no money values are given for the feeds used or for the labor. A calculation based upon current prices will give this information if desired. A summary of the results is given in table 1.

### THE COST OF FEEDING HERD SIRES ON MINNESOTA FARMS

The cost of feeding herd sires on dairy farms located in one of the older and better established dairy sections of Minnesota has been compiled from data secured from a statistical route operated

TABLE 1

*Feed used and cost of keeping a bull for a year*

| | VERMONT | INDIANA | WASHINGTON | LOUISIANA | WEIGHTED AVERAGE |
|---|---|---|---|---|---|
| Number of animals | 27 | 33 | 34 | 24 | |
| Concentrates, pounds | 336 | 1,399 | 630 | 1,202 | 894 |
| Dry roughages, pounds | 6,734 | 4,025 | 5,967 | 779 | 4,544 |
| Succulent roughages, pounds | 2,394 | 6,002 | 3,069 | 1,126 | 3,339 |
| Bedding, pounds | 369 | 645 | 43 | 4 | 255 |
| Labor, hours | 37.7 | 35.7 | 40.4 | 33.6 | 37.08 |
| Pasture | $1.92 | $4.56 | $13.56 | $4.66 | $6.57 |
| Total other costs* | $14.21 | $29.71 | $41.81 | $23.40 | $28.37 |

* "Total other costs" includes interest, insurance, bull's share of buildings, depreciation, etc.

under the supervision of the Division of Agronomy and Farm Management of the University of Minnesota. The object of this route was to collect dependable information on the cost of producing farm products. A state and federal employee visited each farm approximately three times each week and recorded the desired data including the feed fed each animal and established a monthly market price for each feed based on local prices. Yearly feed records taken in this manner are available on 58 mature sires.

The average feed consumption is shown in table 2. Both roughage and concentrates were fed throughout the year. Little

difference is observed between the amount of dry roughage and concentrates consumed during the winter and summer months, the average being approximately 15 pounds of roughage and 4.5 pounds of concentrates per day. As is to be expected the silage feeding was heaviest during the winter months and was generally discontinued in the month of April or May and resumed in late November or December. This table further shows that some of the sires were on pasture during the summer months; the records show that 12 of the 58 sires were so handled.

TABLE 2

*Yearly feed consumption per sire*

Records from 58 animals

| MONTH | DRY ROUGHAGE | CORN SILAGE | CONCENTRATES | PASTURE | TOTAL FEED COST |
|---|---|---|---|---|---|
| | *pounds* | *pounds* | *pounds* | *days* | *dollars* |
| January | 455 | 637 | 137 | 0 | 6.30 |
| February | 381 | 582 | 123 | 0 | 5.88 |
| March | 412 | 585 | 140 | 0 | 6.40 |
| April | 405 | 605 | 108 | 0 | 5.80 |
| May | 418 | 322 | 130 | 4.6 | 5.69 |
| June | 361 | 15 | 138 | 6.2 | 4.96 |
| July | 421 | 95 | 92 | 5.3 | 4.67 |
| August | 443 | 106 | 100 | 5.3 | 5.10 |
| September | 496 | 25 | 156 | 3.6 | 5.86 |
| October | 477 | 135 | 179 | 2.7 | 6.24 |
| November | 564 | 251 | 124 | 0.5 | 6.19 |
| December | 553 | 644 | 140 | 0 | 6.62 |
| Total | 5,386 | 4,002 | 1,567 | 28.2 | 69.71 |

The average grain ration fed, 1567 pounds per animal included corn 127 pounds, small grains 1375 pounds, commercial feeds 47 pounds and linseed oilmeal 18 pounds. Corn, commercial feeds and oilmeal were fed sparingly while the small grains, which were largely oats and barley constituted most of the concentrates. The dry roughage fed the 58 animals included alfalfa, mixed, and prairie hay and corn fodder. In a few cases a limited amount of straw was also used. The value of the feed at current local market was $69.71 per year, although fluctuations due to season

and locality necessarily minimize the value of these figures. If the other expenses incurred in keeping these bulls are assumed to be $28.37, the same as found as an average for the 118 animals included in the study by the United States Department of Agriculture the total average expense involved in maintaining these bulls was $98.08 yearly. In addition about 37 hours of man labor are necessary which may be added at current rates to obtain the total cost involved.

### THE SIRE AS A FACTOR IN THE COST OF MILK PRODUCTION

Sire cost is clearly a factor of some importance in the maintenance of dairy herds. The figures given show that the cost of keeping a bull in recent years has been approximately $100.00 per year in addition to labor. In some cases there is an appreciation in value of the animal as the result of growth or an income from service fees. At most, these possibilities will seldom reduce materially the expense incurred by the bull.

Where the herd is of typical size—10 to 15 cows, it is evident sire service becomes an important factor in the expense of raising heifers to maintain the herd. Assuming an 80 per cent calf crop, a herd of 15 cows will produce 6 heifers a year. For this number the sire cost will be $16.60 each. Unquestionably this sum is fully justified if the heifers raised have the heredity to become profitable dairy animals. The surprisingly high figure undoubtedly explains in part the reluctance of many dairy farmers to make use of improved sires. It also emphasizes the value of bull associations which make possible the use of high class sires at a minimum expense.

### FEED REQUIREMENTS OF BULLS OWNED BY THE UNIVERSITY OF MINNESOTA

Feed records were kept of six mature bulls in the University herd. Weights were taken three days each month. The rations fed included alfalfa hay, corn silage and a grain mixture made up by weight of 200 pounds of ground oats, 100 pounds of ground corn and 100 pounds of ground barley. For a short period during

the summer green feeds were fed including sweet clover, oats and peas and fodder corn. A limited amount of mangels were also fed during a brief period. No attempt was made to feed the animals according to any feeding standard. They were given as much hay as they would clean up, silage to the amount of approximately ten pounds daily, and sufficient grain to keep them in what was considered good breeding condition. The changes in weight were slight during the experimental periods; in fact the weights at the beginning and end of the experimental periods show no wider range than variations from day to day with the same animals.

In table 3 are given the totals for the feed consumed during the experimental periods. Space will not allow more detailed presentation. The following statement of typical rations for summer and winter will supply some details that may be of interest. When green feed was used it replaced the silage.

*Typical daily rations*

| | ALFALFA HAY | CORN SILAGE | GRAIN |
|---|---|---|---|
| | *pounds* | *pounds* | *pounds* |
| Winter: | | | |
| Jerseys | 12 | 8 | 4.5 |
| Holsteins | 18 | 14 | 5.0 |
| Ayrshires | 16 | 12 | 5.0 |
| Summer: | | | |
| Jerseys | 12 | 5 | 5.0 |
| Holsteins | 14 | 13 | 5.0 |
| Ayrshires | 12 | 12 | 5.0 |

The cost of the feed is not given in detail on account of the limited value of such figures due to local and seasonal variations. It may be said however that at local prices the average feed cost was approximately the same as that found in the cost accounting routes, data from which are given in table 2.

The digestible crude protein and total digestible nutrients received in these rations were calculated by using average figures for the feeds included as given by Henry and Morrison (2a). The results are given in table 4, and compared with the estimated

maintenance requirements of animals of the weights represented calculated according to the figures given by the same authors (2b).

TABLE 3

*Feed consumed by mature bulls*

| ANIMAL | LENGTH OF PERIOD | WEIGHT | ALFALFA HAY | CORN SILAGE | GRAIN | GREEN FEED |
|---|---|---|---|---|---|---|
| | *days* | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* |
| Jersey A | 365 | 1,330 | 3,673 | 753 | 1,652 | 2,900 |
| Jersey B | 365 | 1,250 | 4,575 | 3,647 | 1,487 | |
| Jersey C | 90 | 1,452 | 1,277 | 910 | 290 | |
| Holstein A | 365 | 2,510 | 6,550 | 3,420 | 2,146 | 1,891 |
| Holstein B | 153 | 2,310 | 2,494 | 1,836 | 687 | |
| Ayrshire A | 365 | 1,910 | 5,204 | 3,414 | 1,785 | 1,518 |

TABLE 4

*Digestible nutrients consumed daily compared to maintenance requirement according to feeding standard*

| | WEIGHT | NUTRIENTS RECEIVED | | MAINTENANCE REQUIREMENT BY FEEDING STANDARD | | TOTAL DIGESTIBLE NUTRIENTS RECEIVED COMPARED TO FEEDING STANDARD |
|---|---|---|---|---|---|---|
| | | Digestible crude protein | Total digestible nutrients | Digestible crude protein | Total digestible nutrients | |
| | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* | *per cent* |
| Jersey A | 1,330 | 1.64 | 9.9 | 0.93 | 10.5 | 91 |
| Jersey B | 1,250 | 1.81 | 11.3 | 0.87 | 9.9 | 114 |
| Jersey C | 1,452 | 1.90 | 11.5 | 1.02 | 11.5 | 100 |
| Holstein A | 2,510 | 2.67 | 16.0 | 1.76 | 19.9 | 80 |
| Holstein B | 2,310 | 2.26 | 13.9 | 1.62 | 18.3 | 76 |
| Ayrshire A | 1,910 | 1.77 | 14.0 | 1.34 | 15.1 | 92 |

It will be noted that four of the six bulls required less total digestible nutrients than the amount estimated from the feeding standard. Only one used more than the estimated nutrients. Thus results indicate that the bull offers no special problems so far as feed requirements are concerned. There is indeed a sug-

gestion of a maintenance requirement slightly lower than that ordinarily assumed for cows. The number of animals is too limited to justify any definite conclusions to that effect. It seems safe to conclude from the data that the ordinary maintenance requirements for total digestible nutrients may be assumed in connection with the feeding of mature bulls. The data gives no basis for estimating the protein requirements of the animal or the possible influence of the character of the ration upon the breeding qualities of the animal.

## RATIONS FOR GROWING BULLS

The cost of raising a bull to maturity is a question of some importance. Although our records do not furnish complete data on this subject they do furnish some facts worth recording. Records were kept of the feed received and the gains in weights of four bulls representing four breeds, and between the age of one and four years. The ration used for these growing animals was the same in character as that supplied the mature animals. They were allowed all the roughage they would consume and in addition it was necessary to give them a liberal grain allowance in order to obtain what was considered a normal rate of growth. Two of them were fed more grain per day than the mature bulls received.

The animals included were as follows:

| | AGE AT BEGINNING | | LENGTH OF PERIOD | WEIGHT AT BEGINNING | GAIN PER DAY |
|---|---|---|---|---|---|
| | *years* | *months* | *days* | *pounds* | *pounds* |
| Guernsey A | 2 | 5 | 365 | 1,285 | 0.80 |
| Guernsey B | | 8 | 92 | 334 | 1.20 |
| Jersey D | | 10 | 92 | 539 | 1.30 |
| Ayrshire B | | 10 | 212 | 448 | 1.52 |

The average feed received per day is given in table 5.

It is of interest to compare the nutrients received with the allowance that would be made by Morrison's feeding standard (2c). The weight of the larger Guernsey was beyond the range of the Morrison table and is for this reason omitted.

*Nutrients received by growing bulls in percentage of Morrison's feeding standard*

| | DIGESTIBLE CRUDE PROTEINS | TOTAL DIGESTIBLE NUTRIENTS |
|---|---|---|
| Guernsey B | 102 | 103 |
| Jersey D | 117 | 101 |
| Ayrshire B | 98 | 85 |

These figures although few in number indicate that bulls fed according to accepted practices in well-managed dairy herds will ordinarily receive approximately the allowance of nutrients called for by a widely used feeding standard.

TABLE 5

*Feed received per day by growing bulls*

| | WEIGHT | ALFALFA HAY | CORN SILAGE | GRAIN | GREEN FEED |
|---|---|---|---|---|---|
| | *pounds* | *pounds* | *pounds* | *pounds* | *pounds* |
| Guernsey A | 1,285 | 16.6 | 8.3 | 6.5 | 6.7 |
| Guernsey B | 334 | 5.4 | 4.0 | 4.0 | 0 |
| Jersey D | 539 | 7.7 | 5.9 | 5.0 | 0 |
| Ayrshire B | 448 | 6.7 | 5.8 | 4.3 | 0 |

## SUMMARY

A compilation from figures published by the United States Department of Agriculture gives the average feed received by 118 bulls on farms in four sections of the United States. In addition 37 hours man-labor were required, pasture to the amount of $6.57, and other cost totaling $28.37.

A summary is given of the feed received by 58 bulls on Minnesota farms over a period of a year. The average feed cost at current local prices was $69.71. Assuming the same other costs as found by the United States Department of Agriculture, the total cost of keeping a mature bull is approximately $98.00 a year in addition to about 37 hours of man labor.

The amount of feed consumed by six mature bulls in the University of Minnesota herd is given. The total digestible

nutrients contained approximate closely the maintenance requirements as estimated by the use of feeding standards.

The nutrients received by growing bulls fed according to common practice on dairy farms were close to the amounts prescribed by Morrison's feeding standard for growing dairy cattle of the weights represented.

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# NORMAL VARIATIONS IN THE INORGANIC PHOSPHORUS OF THE BLOOD OF DAIRY CATTLE*

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The inorganic phosphate of the blood has been studied extensively in recent years because of its relation to various problems of nutrition, metabolism, and disease. In cattle the effect of the phosphorus level of the food on the percentage of inorganic phosphorus of the blood has already been demonstrated by us (1) (2). During the collection of these data we were impressed by the fact that unaccountable, and frequently large fluctuations may occur from day to day. This result led us to adopt the mean of the blood phosphate values obtained on three consecutive days as the probable true value. The day-to-day fluctuations, however, remained unexplained.

The physiological factors influencing the concentration of blood phosphate have not been studied extensively. Havard and Reay (3) seem to have been the only workers who have noted marked periodic fluctuations. They report that the concentration of blood phosphate is very unsteady in human blood studied at hourly intervals when the subject is not kept completely at rest. Ordinary movements of the subject about the laboratory cause large variations. Havard and Reay believe that these changes are the result of exercise which they later (4) showed causes first a small rise and then a rapid fall in blood phosphate. In these studies the lowest level was reached in about three-quarters of an hour after the period of exercise. Harrop and Benedict (5), working with rabbits, observed that convulsions following

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strychnine administration resulted in great increase in blood phosphate. Short, vigorous exercise causes an increase in blood pressure, according to the work of Young and coworkers (6). Scheunert and Müller (7) found with horses that vigorous running causes a marked loss in $CO_2$-combining capacity. The effects noted on blood pressure and $CO_2$-combining capacity may be coincident with, rather than related to the effects on blood phosphate.

A number of workers (5) (8) (9) (10) have noted that ingestion of soluble carbohydrates or the administration of insulin causes a decrease in blood phosphate. These results establish the intimate association between blood phosphate and glucose metabolism and support the belief (4) (5) that the effect of exercise on blood phosphate is directly related to this association.

The relation of blood dilution to phosphate concentration must be considered. Although no studies seem to have been made on the inorganic phosphorus, Priestley (11) found that water drinking under controlled conditions causes a slight but definite decrease in chlorides and dry matter. Greene and Rowntree (12) administered excessive amounts of water to dogs and secured dilution of the blood. Marx (13) found a marked dilution of the hemoglobin within 15 to 20 minutes after a man had drunk two liters of water or tea.

Meigs and Blatherwick (14) noted that the inorganic phosphorus is likely to be low in mature cows just after calving. They conclude that it seems to depend to some extent on the amount of grain fed. Robinson and Huffman (15) in an extensive study found that in nearly every case, the inorganic phosphorus of the blood of the dam is below that of the calf at birth, but that the values for the maternal inorganic phosphorus usually rise during six to eighteen hours after calving to a rather definite maximum which is followed by a depression. Beyond this the fluctuations are irregular. In their work with calves, Meigs, Blatherwick, and Cary (16) found the inorganic phosphorus of the plasma to be fairly high in new born calves but tending to increase for some time and reaching a maximum at about the age of six months.

## EXPERIMENTAL

The experimental results fall into two groups. The first group comprises the normal variations in blood phosphate from day to day in samples taken at the same hour on three consecutive days. Some variations encountered at periodic intervals on the same day are also reported. The second group consists of the results of attempts to determine the extent to which such physiological factors as eating, drinking, and exercise affect the blood phosphate of cattle. It was hoped that these factors would throw light on the daily variations encountered. In addition, data are presented on the influence of parturition and age on the inorganic phosphate in the blood.

The blood used in the analyses was drawn from the jugular vein, and allowed to flow into 100 cc. glass tubes, containing 1 cc. of a saturated solution of sodium citrate. The size of sample drawn was 50 cc. The blood was mixed, thoroughly, with the sodium citrate solution immediately to prevent coagulation. It was then centrifuged to separate the plasma from the corpuscles. Upon completion of the centrifugation, sufficient plasma for the test was pipetted off, and preserved in an ice box until tested. In nearly all cases the analyses involving single samples were completed the same day the blood sample was drawn. Where the nature of the study permitted, the test was made on a composite sample of plasma taken from blood drawn at the same hour on three consecutive days. In some of the work in the second group of experiments, however, composite samples could not be used, and individual samples of plasma were analyzed for inorganic phosphorus.

### *Method of analysis*

The Briggs (17) modification of the Bell-Doisy colorimetric method of phosphate analysis was used. All tests were made in duplicate and the averages were used in all comparisons.

### *Animals used*

Twenty-five different animals furnished the data for the first group. They were all in the University experimental herd.

TABLE 1

*Day-to-day variations in inorganic phosphate in successive three-day periods per 100 cc. plasma*

| ANIMAL | PERIOD 1 | | | | | | PERIOD 2 | | | | | | PERIOD 3 | | | | | | PERIOD 4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $P_1$ | | $P_2$ | | $P_3$ | | $P_1$ | | $P_2$ | | $P_3$ | | $P_1$ | | $P_2$ | | $P_3$ | | $P_1$ | | $P_2$ | | $P_3$ | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| E 33 | 3.06 | 3.12 | 2.37 | 2.54 | 2.07 | 2.12 | 2.55 | 2.57 | 1.96 | 1.98 | 2.08 | 2.08 | 2.31 | 2.31 | 3.33 | 3.37 | 3.29 | 3.33 | 1.74 | 1.89 | 1.67 | 1.67 | 1.75 | 1.75 |
| E 73 | 2.26 | 2.37 | 2.72 | 2.76 | 2.28 | 2.40 | 1.80 | 1.84 | 2.44 | 2.51 | 2.01 | 2.02 | 2.43 | 2.45 | 2.19 | 2.19 | 2.19 | 2.21 | 2.35 | 2.35 | 1.71 | | 1.90 | |
| E 74 | 5.04 | 5.04 | 5.74 | 5.88 | 5.65 | 5.71 | 4.25 | 4.25 | 5.49 | 5.55 | 5.74 | | 5.49 | 5.49 | 5.26 | 5.26 | 5.43 | 5.49 | 4.80 | 4.80 | 4.64 | 4.67 | 4.80 | 4.80 |
| E 75 | 1.72 | 1.79 | 1.82 | 1.86 | 1.59 | 1.72 | 1.79 | 1.93 | 1.84 | 1.97 | 1.61 | 1.63 | 1.90 | 1.93 | 2.21 | 2.23 | 1.69 | 1.71 | 2.45 | 2.56 | 2.14 | 2.24 | 2.39 | 2.50 |
| E 91 | 2.29 | 2.33 | 2.24 | 2.29 | 2.51 | 2.63 | 1.77 | 1.85 | 1.94 | 1.95 | 2.04 | 2.04 | 1.00 | 1.01 | 1.52 | 1.52 | 1.25 | 1.26 | 0.88 | 0.88 | 1.17 | 1.17 | 1.06 | 1.06 |
| E 92 | 3.79 | 4.03 | 4.27 | 4.35 | 5.00 | 5.00 | 3.70 | 3.78 | 3.85 | 3.95 | 8.33 | 8.33 | 5.20 | 5.20 | 4.83 | 4.87 | 5.63 | 5.69 | 6.71 | 6.71 | 6.53 | 6.53 | 4.86 | 4.89 |
| E 93 | 1.07 | 1.09 | 0.93 | 0.95 | 1.20 | | 2.12 | | 1.97 | 2.02 | 1.91 | 1.96 | 0.96 | | 1.23 | 1.24 | 0.89 | 0.91 | 1.53 | 1.53 | 1.90 | 2.00 | 1.25 | 1.29 |
| E 94 | 1.29 | 1.31 | 1.13 | 1.14 | 1.40 | 1.40 | 2.27 | | 2.17 | 2.42 | 1.56 | 1.56 | 1.73 | 1.77 | 1.27 | 1.29 | 0.91 | 0.92 | 1.13 | 1.21 | 0.84 | 0.84 | 1.25 | 1.29 |
| E 84 | 9.09 | 9.25 | 7.14 | 7.24 | 7.09 | 7.09 | 4.96 | 4.99 | 5.49 | 5.55 | 5.37 | 5.37 | 6.06 | 6.06 | 6.21 | 6.25 | 6.52 | 6.61 | | | | | | |
| E 58 | 7.04 | 7.14 | 6.91 | 7.04 | 5.54 | 5.67 | 4.05 | 4.11 | 5.43 | 5.49 | 6.75 | 6.75 | | | | | | | | | | | | |
| E 68 | 6.62 | 6.71 | 6.75 | 6.85 | 6.57 | 6.71 | 5.59 | 5.67 | 6.45 | 6.53 | 6.95 | 7.04 | | | | | | | | | | | | |
| E 77 | 8.07 | 8.19 | 8.13 | 8.13 | 8.77 | 8.93 | 7.09 | 7.09 | 6.90 | 6.90 | 6.21 | 6.25 | | | | | | | | | | | | |
| E 80 | 7.04 | 7.24 | 6.85 | 6.85 | 6.85 | 6.85 | 6.75 | 6.85 | 6.67 | 6.75 | 6.25 | 6.49 | | | | | | | | | | | | |
| E 81 | 9.43 | 9.52 | 8.00 | 8.06 | 8.06 | 8.33 | 7.70 | 7.70 | 7.14 | 7.14 | 7.30 | 7.40 | | | | | | | | | | | | |
| E 83 | 7.46 | 7.57 | 6.25 | 6.45 | 6.67 | 6.67 | 7.14 | 7.14 | 7.40 | 7.40 | 7.09 | 7.19 | | | | | | | | | | | | |
| E 85 | 9.52 | 9.90 | 7.24 | 7.24 | 8.06 | 8.26 | 7.86 | 7.99 | 7.14 | 7.14 | 7.40 | 7.46 | | | | | | | | | | | | |
| E 86 | 8.13 | 8.13 | 7.75 | 7.87 | 7.63 | 7.63 | 7.30 | 7.40 | 7.45 | 7.49 | 6.57 | 6.57 | | | | | | | | | | | | |
| E 88 | 9.52 | 9.52 | 7.30 | 7.40 | 8.06 | 8.13 | 7.81 | 8.93 | 7.51 | 7.51 | 8.26 | 8.26 | | | | | | | | | | | | |
| E 89 | 8.33 | 8.33 | 7.57 | 7.93 | 7.81 | 7.81 | 7.80 | 7.99 | 7.45 | 7.56 | 6.80 | 6.90 | | | | | | | | | | | | |
| E 78 | 7.69 | 7.80 | 6.45 | 6.45 | 6.17 | 6.33 | | | | | | | | | | | | | | | | | | |
| E 79 | 6.90 | 6.90 | 6.90 | 6.90 | 6.49 | 6.49 | | | | | | | | | | | | | | | | | | |
| E 87 | 8.93 | 9.09 | 8.48 | 8.62 | 10.41 | 10.63 | | | | | | | | | | | | | | | | | | |
| E 95 | 7.34 | 7.45 | 6.40 | 6.40 | 7.51 | 7.75 | | | | | | | | | | | | | | | | | | |
| E 96 | 9.70 | 9.89 | 8.48 | 8.48 | 8.61 | 8.76 | | | | | | | | | | | | | | | | | | |

The ages of the animals ranged from calves to mature cows, some of the latter being in milk. The animals represented various planes of phosphorus intake.

All the animals used in the second group, with the exception of two cows, were from the general University herd, and were being fed the usual herd rations. The two cows excepted were from the University experimental herd. They were both receiving either normal rations or a phosphorus-deficient ration plus a phosphate supplement. Yearling heifers fed normal rations were used for the study of the feeding and drinking factors. In the study of the age factor, calves from the general herd consisting of Holstein-Friesian, Jersey, and Guernsey breeds were used. Their feed while young consisted of milk, but alfalfa hay and cracked corn were given as soon as they would eat them.

### DAY-TO-DAY VARIATIONS

The data collected comprise 60 sets of three-day samples, taken at monthly intervals from August to December, 1926. Five animals had only one period of 3 consecutive days, 10 animals had 2 periods, 1 animal 3 periods, and 8 animals 4 periods. The complete data, including the duplicate analyses of each sample are given in table 1. In this table P is the milligrams of inorganic phosphorus per 100 cc. of plasma, the subscripts 1, 2, and 3 referring to the first, second, and third days, and the subscripts *a* and *b* to the duplicate analyses.

The coefficients of correlation between the respective days, calculated by the formula of Harris (18) are as follows:

$$r_{P_1P_2} = +0.970033 \pm 0.005141$$
$$r_{P_1P_3} = +0.930870 \pm 0.011623$$
$$r_{P_2P_3} = +0.954174 \pm 0.007798$$

It is apparent that there is a high correlation between the values obtained on successive days when the data are considered as a whole. However, simple inspection of the data in table 1 shows that marked variations from the general law are of frequent occurrence. In order to secure data showing to what extent the actual values deviate from the theoretical values indicated

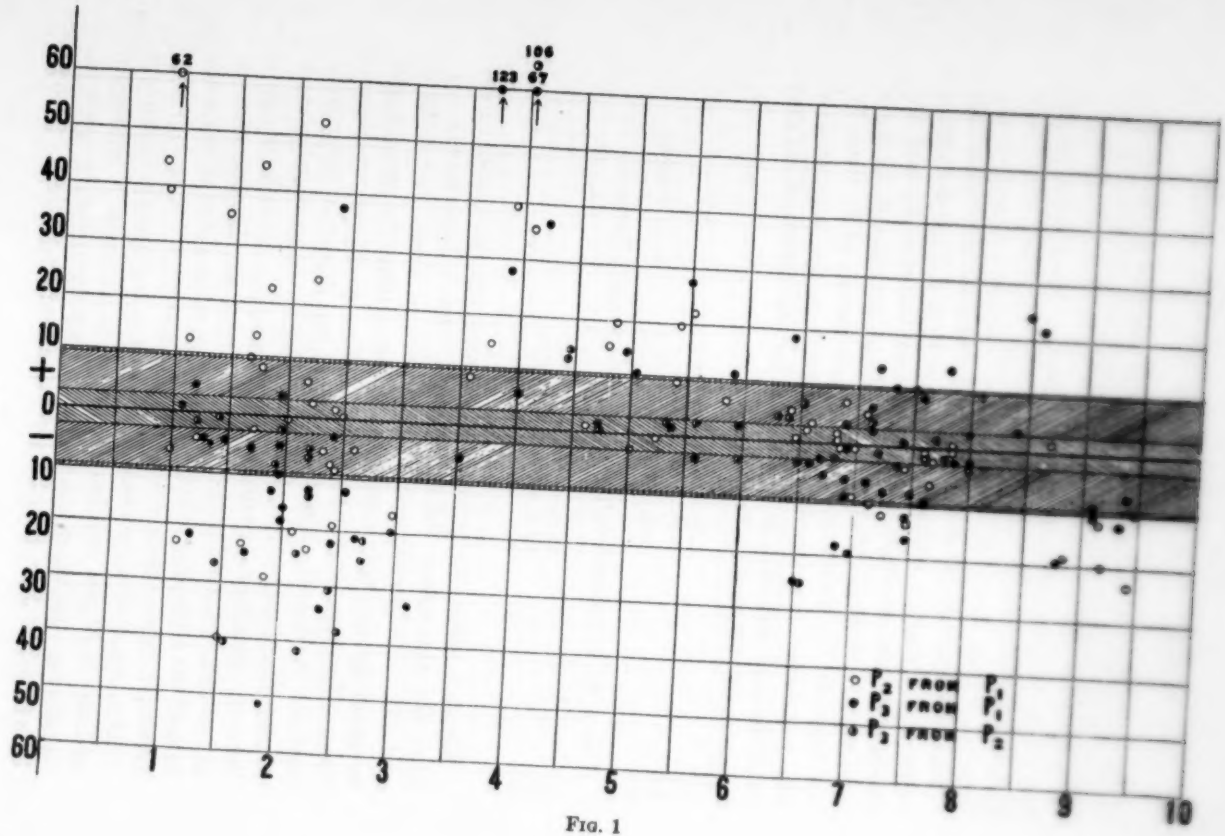
62
106
123
67
60
50
40
30
20
10
+
0
−
10
20
30
40
50
60
1
2
3
4
5
6
7
8
9
10
$P_2$ FROM $P_1$
$P_3$ FROM $P_1'$
$P_3$ FROM $P_2$

FIG. 1

by the coefficient of correlation, use was made of Harris' modification of the regression formulae, as follows:

(1) $$P_2 = (\bar{P}_2 - r_{P_1P_2}\bar{P}_1) + r_{P_1P_2}P_1$$
(2) $$P_3 = (\bar{P}_3 - r_{P_1P_3}\bar{P}_1) + r_{P_1P_3}P_1$$
(3) $$P_3 = (\bar{P}_3 - r_{P_2P_3}\bar{P}_2) + r_{P_2P_3}P_2$$

Thus, by formula (1) it is possible to calculate the theoretical value of $P_2$ using $P_1$, the mean values of $P_1$ and $P_2$ of the entire series, and the coefficient of correlation between $P_1$ and $P_2$. Similarly, formula (2) makes it possible to calculate $P_3$ from $P_1$, and formula (3) makes possible the calculation of $P_3$ from $P_2$. The calculation is a simple one, inasmuch as the parenthetical value in each equation is a constant for the equation, so that formulae (1), (2), and (3) become

(1) $$P_2 = K_1 + r_{P_1P_2}P_1$$
(2) $$P_3 = K_2 + r_{P_1P_3}P_1$$
(3) $$P_3 = K_3 + r_{P_2P_3}P_2$$

Using the calculated value for $K$, the formulae become

(1) $$P_2 = -0.042 + 0.970\ P_1$$
(2) $$P_3 = +0.256 + 0.931\ P_1$$
(3) $$P_3 = +0.322 + 0.954\ P_2$$

Figure 1 shows the percentage variations of the actual values from the theoretical values obtained from equations (1), (2), and (3). The physiological significance of these variations is revealed by the points outside of and within the cross-hatch lines. The lines drawn at +10.37 and −10.37 per cent represent the maximum analytical error of the duplicate determinations on the same sample of plasma. All the points outside these lines are therefore physiologically significant. The lines drawn at +3 and −3 per cent were arbitrarily chosen because calculation of the analytical errors showed that 85 per cent of the mean values of duplicate analyses had an error of 3 per cent or less. This means that 85 per cent of the points between the 3 per cent and the 10.37 per cent lines represent physiologically significant variations. In like manner 15 per cent of the points between the 0 line and the 3 per cent lines are significant variations. A sum-

mation of the significant variations of the actual from the theoretical values shows a total of 137.55 out of 180, or 76.4 per cent.

It was the striking day-to-day variations of the inorganic phosphate in the blood of single cows that prompted the major portion of the second group of experiments. It seems probable that the inorganic phosphorus content of the blood plasma of cows reported in the literature, which apparently represent single random samples, are very likely incorrect. In fact, our data indicate that there is only about one chance in four of such a procedure giving a correct result.

TABLE 2

*Inorganic blood phosphate variations at two-hour intervals during the day*

| TIME | COW NUMBER | P PER 100 CC. PLASMA | |
|---|---|---|---|
| | | (a) | (b) |
| | | *mgm.* | *mgm.* |
| 9:00 a.m. | E 75 | 5.15 | 5.15 |
| 11:00 a.m. | E 75 | 5.10 | 5.05 |
| 1:00 p.m. | E 75 | 4.16 | |
| 3:00 p.m. | E 75 | 4.16 | 4.24 |
| 5:00 p.m. | E 75 | 5.10 | 5.10 |
| 9:00 a.m. | E 73 | 1.41 | 1.41 |
| 11:00 a.m. | E 73 | 1.98 | 1.96 |
| 1:00 p.m. | E 73 | 1.96 | 1.96 |
| 3:00 p.m. | E 73 | 1.52 | 1.54 |
| 5:00 p.m. | E 73 | 1.60 | 1.56 |

Numerous physiological factors might be responsible for these variations, most of which are impractical to control experimentally. Their influence cannot be determined at present.

Blood was drawn from two dry cows at two-hour intervals, beginning at 9:00 a.m., to determine whether the inorganic phosphorus varies appreciably at such intervals. Each cow was in her stall and allowed water *ad libitum* but was not fed. The results, given in table 2, show a marked depression followed by a rise in the case of one animal and a moderate rise followed by a depression in the case of the other animal. These variations resemble those found by Havard and Reay (3) to occur in humans.

The three physiological factors which can be readily controlled experimentally and which from the review of the literature, may be expected to exert an influence on the percentage of inorganic phosphorus of the blood are eating, drinking, and exercise. The latter might be especially significant in the case of animals that struggle violently before the blood sample can be taken.

TABLE 3

*Effect of feeding on inorganic phosphate in blood plasma of cattle*

| COW NUMBER | PLASMA P BEFORE FEEDING | CHANGES IN PLASMA P | | | |
|---|---|---|---|---|---|
| | | Three-fourths hour after feeding | One and three-fourths hours after feeding | Two and three-fourths hours after feeding | Five hours after feeding |
| | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| 402 | 6.41 | +0.68* | | −0.42* | −0.50* |
| 402 | 6.49 | +0.80* | +0.08 | −0.59* | |
| 402 | 5.83 | +0.46* | +0.08 | −0.12 | |
| 403 | 7.14 | −0.10 | | −0.02 | +0.17* |
| 403 | 8.13 | +0.49* | −0.07 | −0.49* | |
| 403 | 7.04 | +0.31* | +0.23* | −0.28* | |
| 175 | 6.54 | +0.08 | +0.40* | −0.22* | |
| 176 | 6.94 | +0.52* | −0.11 | −0.31* | |
| 176 | 6.49 | −0.28* | +0.18* | −0.80* | |
| 174 | 6.62 | −0.13 | −0.08 | −0.26* | |

*Difference between duplicate analyses*

| | mgm. |
|---|---|
| Mean | 0.043 |
| Maximum | 0.14 |
| Minimum | 0.00 |

* Significant change.

## INFLUENCE OF FEEDING

Ten trials were run to study the effect of feeding on the inorganic blood phosphate. Blood samples were drawn before feeding in the morning and again at intervals of 45 minutes, 1.75 hours, and 2.75 hours, and in 2 cases 5 hours after feeding. The animals were kept in the stall continuously both prior to and after feeding. Water was allowed *ad libitum* from drinking fountains in the stalls. The feed was the same as used in the herd for growing heifers and was considered a normal ration.

Table 3 gives the results of the various trials and shows the inorganic plasma phosphate before feeding and the consecutive milligram changes at the various intervals after feeding. The significance of the changes has been interpreted after subtracting the maximum analytical difference obtained in duplicate analyses of the same sample. This is the most rigid interpretation possible. On this basis it is seen that a significant rise in blood phosphate had taken place by three-fourths of an hour after feeding in 6 of the 10 trials, and a significant fall in one trial, with no significant change in the other 3 trials. In one of the latter trials, a significant rise had occurred by the end of 1.75 hours, and in another there was a significant fall which was not manifested until the end of 2.75 hours.

There are not enough significant changes at the end of 1.75 hours to indicate any single physiological effect. At the end of 2.75 hours, however, a significant fall had occurred in eight of the ten trials in comparison with the inorganic blood phosphate the previous hour.

The mean magnitude of significant change in blood phosphate at the end of the 0.75 hour interval over and above the maximum analytical difference amounts to 0.363 mgm. or 5.7 per cent of the mean blood phosphate in these cases. This is not a great effect but is sufficient to warrant consideration in determining a procedure for taking blood samples from dairy cattle.

## INFLUENCE OF DRINKING

Six trials were run to study the effect of drinking by cattle on the inorganic phosphorus content of the blood. The animals were first deprived of water, for various lengths of time in the different trials, to insure the drinking of water at the time it was desired to conduct the test. In the first and second trials, the animals were deprived of water for 24 hours, in the third and fourth trials for 30 hours, and in the fifth and sixth trials, they were deprived of water for 44 hours to insure copious drinking. Blood samples were drawn in each case just before the animals were watered, and again at 2 thirty-minute intervals after they had finished drinking. As shown in table 4, there was a decrease

in blood phosphate in every case 30 minutes after drinking. In only 2 cases, however, was there any further decrease one hour after drinking.

The maximum analytical error in these analyses was the same as in the experiments on the effect of feeding, namely, 0.14 mgm. Applying this figure in the same manner as in the experiments on the effects of feeding shows that 5 of the 6 trials give a significant fall in blood phosphate after drinking. However, the mean percentage variation of the significant change is small, amounting

TABLE 4

*Effect of water drinking on the inorganic phosphate in blood plasma of cattle*

| COW NUMBER | TIME DEPRIVED OF WATER | WATER DRUNK | PLASMA P BEFORE DRINKING | CHANGE IN PLASMA P | |
|---|---|---|---|---|---|
| | | | | One-half hour after drinking | One hour after drinking |
| | *hours* | *pounds* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| 402 | 24 | | 7.14 | −0.10 | 0.00 |
| 402 | 30 | 40 | 5.57 | −0.33* | +0.14 |
| 402 | 40 | 59 | 6.25 | −0.52* | +0.15 |
| 403 | 24 | | 8.77 | −0.30* | −0.78* |
| 403 | 30 | 43 | 7.35 | −0.38* | +0.17* |
| 403 | 44 | 56 | 6.25 | −0.26* | −0.46* |

*Difference between duplicate analyses*

| | mgm. |
|---|---|
| Mean | 0.021 |
| Maximum | 0.14 |
| Minimum | 0.00 |

* Significant change.

to only 3.6 per cent of the value of the blood phosphate before drinking. In view of the fact that the cattle in these experiments drank an excessive amount of water, it is unlikely that the ordinary amounts of water drunk by cattle at any one time will have any significant influence on the true content of inorganic phosphate.

## INFLUENCE OF EXERCISE

Twelve trials were run to study the effects of exercise on the inorganic blood phosphorus. In the first 6 trials, heifers about

seven months old, which had a high blood phosphorus content, were used. In trials 7 to 10, inclusive, cows with a medium content of inorganic blood phosphorus were used, while in the eleventh and twelfth trials, yearling heifers were used.

In each case, the animal was bled after having eaten in the morning. It was then turned out into a lot and caused to run for 5 to 10 minutes or until it was breathing hard. It was then re-

TABLE 5

*Effect of exercise on the inorganic phosphate in blood plasma of cattle*

| COW NUMBER | PLASMA P BEFORE EXERCISE | CHANGES IN PLASMA P | | | |
|---|---|---|---|---|---|
| | | At once | One-half hour after exercise | One hour after exercise | Two hours after exercise |
| | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| 179 | 8.77 | +0.40* | −2.50* | | |
| 179 | 7.25 | +0.44* | −0.84* | −0.18* | +0.58* |
| 179 | 8.00 | +0.47* | −1.48* | −0.54* | +0.09 |
| 554 | 8.66 | −0.19* | −1.91* | | |
| 554 | 8.06 | +0.07 | −0.73* | +0.10 | +0.30* |
| 554 | 8.89 | +0.28* | −1.11* | −0.19* | +0.13 |
| 75 | 4.26 | +1.30* | −2.23* | −0.39* | +0.09 |
| 75 | 4.08 | +0.14 | −0.60* | −0.52* | −0.31* |
| 93 | 4.06 | +0.19* | −1.49* | +0.77* | −0.04 |
| 93 | 4.35 | +0.75* | −1.40* | +0.65* | −0.26* |
| 402 | 5.95 | +0.75* | −1.08* | +0.12 | +0.07 |
| 403 | 8.06 | +0.71* | −0.77* | −1.01* | −0.19* |

*Difference between duplicate analyses*

| | mgm. |
|---|---|
| Mean | 0.034 |
| Maximum | 0.17 |
| Minimum | 0.00 |

* Significant change.

turned to the stall and bled as quickly as possible. Usually, about fifteen minutes elapsed between the first and second bleedings. The animal was then allowed to stand quietly in the stall, and was re-bled at one-half-hour, one-hour, and two-hour intervals. In the first 2 trials, the one-hour and two-hour intervals were omitted.

The results of the study are given in table 5. They show a

marked increase of inorganic phosphate just after exercise, followed by a still more marked decrease at the half-hour, and in most cases a still further, but less marked decline after a further half-hour.

The relative significance of these changes after subtracting the maximum analytical difference between duplicate analyses is striking. Ten of the 12 trials gave a significant rise in inorganic phosphate immediately after the period of exercise, and in every trial a significant fall had occurred at the end of one-half-hour. In 6 out of 10 trials there was a further significant fall after another half-hour interval, while 2 trials gave a significant rise after this period. At the two-hour interval after the exercise apparently all but one of the animals had either attained the maximum after-effects of the exercise or had partially recovered. Animal 75 (second test), which apparently showed the after-effects of the exercise more slowly than the other animals still exhibited a further fall in blood phosphate at this time. The figure for animal 403 at the two-hour period is not greatly in excess of 0.17 mgm., the analytical error applied.

Viewed from a percentage standpoint the mean significant changes are as follows. Immediately after exercise there was a rise of 6.34 per cent compared with the value before exercise. One-half hour later there was a fall of 22 per cent compared with the value immediately after exercise. After another half-hour there was a further drop of 4.9 per cent, using the first half-hour value as a basis.

It is evident that the activity of the animal is a very significant factor in modifying the inorganic phosphate of the blood. Some of the variations occurring with individual cows were almost as great as some of the day-to-day variations encountered. For example, in the first test with animal 75 there was first a 30.5 per cent increase followed by a 40 per cent decrease. Apparently every possible precaution should be taken to avoid unnecessary activity in drawing blood samples from cattle for estimation of inorganic phosphate content.

## INFLUENCE OF PARTURITION

Opportunity was presented to study the influence of 2 other normal physiological factors on inorganic blood phosphorus, namely, the effect of parturition and of age. Obviously it was not expected that such data would throw any light on the day-to-day variations.

TABLE 6

*Changes occurring in the inorganic phosphorus content of the blood of cows at parturition*

| DAYS FROM CALVING | INORGANIC PHOSPHORUS IN 100 CC. OF PLASMA | | | | | |
|---|---|---|---|---|---|---|
| | No. 75 | No. 81 | No. 93 | No. 407 | No. 409 | No. 547 |
| Before | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 3 or more | 4.79 | 4.40 | 4.16 | 5.32 | | 4.84 |
| 2 | | | 4.89 | 5.43 | 4.50 | 4.95 |
| 1 | | 3.07 | 3.51 | 4.56 | 4.31 | 2.77 |
| 0 (calved) | 2.46 | 3.73 | 3.39 | 2.14 | 4.25 | 2.87 |
| After | | | | | | |
| 1 | 2.64 | 4.22 | 3.83 | 2.17 | 3.33 | 2.69 |
| 2 | 2.81 | 4.74 | 5.10 | 4.07 | 2.51 | 2.72 |
| 3 | 4.92 | 5.36 | 3.79 | 4.17 | 3.47 | 3.70 |
| 4 | | | | | 3.40 | |
| 5 | | 6.22 | 1.67 | 4.35 | | 4.85 |
| 6 | 4.86 | | | | 3.77 | |
| 7 | | 5.84 | | | | |
| 8 | | | 2.06 | 4.33 | | |
| 9 | | 6.49 | | | 4.08 | |
| 10 | | | | | | |
| 11 | | 5.92 | 4.25 | | 5.70 | |

Six cows were used in the study of the effect of parturition. The general plan was to test a three-day composite sample of blood plasma, about one week before parturition, then take daily samples of blood so that individual samples could surely be tested on the 3 days immediately preceding calving. On account of certain cows calving sooner than expected, incomplete records were secured in those cases. In general, the results showed there was not much change in the inorganic phosphorus until less than

3 days before calving. Therefore, in order to simplify the tabulation of the results, all tests made 3 or more days before parturition were averaged, and this figure was taken as the starting point in the tables.

Table 6 shows the results of the study in tabular form. The amount of inorganic phosphorus in the blood on the day of calving is shown between the horizontal lines opposite "0" days. Most

TABLE 7

*Comparison of the inorganic phosphorus content of the blood of the newborn calf with that of the dam*

| DAYS FROM CALVING | INORGANIC PHOSPHORUS IN 100 CC. OF PLASMA | | | | | |
|---|---|---|---|---|---|---|
| | No. 407 | No. 407's calf | No. 409 | No. 409's calf | No. 547 | No. 547's calf |
| Before | mgm. | mgm. | mgm. | mgm. | mgm. | mgm. |
| 3 or more | 5.32 | | | | 4.84 | |
| 2 | 5.43 | | 4.50 | | 4.95 | |
| 1 | 4.56 | | 4.31 | | 2.77 | |
| 0 | 2.14 | 5.13 | 4.25 | 5.08 | 2.87 | 5.43 |
| After | | | | | | |
| 1 | 2.17 | | 3.33 | 6.25 | 2.69 | |
| 2 | 4.07 | 6.13 | 2.51 | 6.49 | 2.72 | 5.73 |
| 3 | 4.17 | 6.76 | 3.47 | | 3.70 | |
| 4 | | | 3.40 | | | |
| 5 | 4.35 | | | | 4.85 | 5.97 |
| 6 | | | 3.77 | 6.90 | | |
| 7 | | 6.90 | | | | 6.10 |
| 8 | 4.33 | | | | | |
| 9 | | | 4.08 | | | |

of the cows showed a tendency to be low in phosphorus at the time of calving. In every case where comparative records were obtained, there was a decrease in inorganic phosphorus on the day before parturition. This decrease from the preceding day varied from 0.2 to about 2.2 mgm. of inorganic phosphorus per 100 cc. of plasma.

The time of lowest inorganic phosphorus in the blood was quite variable. In only 2 cases was it lowest on the day of calving. The other low points were observed on the day preceding par-

turition, on the day following and 2 days after calving, while one, No. 93, had a distinct decrease near the time of calving followed by a rise and then by a decrease much greater than the first, until on the fifth day from calving her blood contained only 1.67 mgm. of inorganic phosphorus per 100 cc. of plasma. This was about 2.5 mgm. below her average value three or more days before calving.

None of the other cows showed any tendency to have a second decrease several days after calving. No. 409, however, continued to decrease until 2 days after parturition when the inorganic phosphorus was 2 mgm. below the value shown 2 days before calving. The amount of decrease in inorganic phosphorus shown by the various cows at or near the time of parturition, varied from about 1.3 to 3.2 mgm. per 100 cc. of plasma. In only one case was the decrease less than 2 mgm.

Three calves were tested for inorganic blood phosphorus at or soon after birth, and at intervals during the first week. Table 7 shows the comparison of the calf blood with that of its dam. In every case, the blood of the calf contained more inorganic phosphorus at birth than that of the dam, and the value compared favorably with that of its mother several days before calving.

### INFLUENCE OF AGE

The calves in the dairy herd, not over 185 days old, all of which were fed on normal rations, were used to study the effect of age on the inorganic phosphorus content of blood. These were classified so that they could be tested at or near their monthly birth dates. The limits of ages for testing were 0 to 7, 25 to 35, 55 to 65, 85 to 95, 115 to 125, 145 to 155, and 175 to 185 days. To make a comparison with animals of older ages, 26 tests, previously made, were tabulated. The older animals were not tested within any particular age limits. The system of age classification used for them was therefore different from that used for the calves under 185 days of age.

The results of this study are shown in table 8. The calves were distinctly lower in the inorganic phosphorus content of the blood during the first week after birth and increased fairly rapidly

up to 2 months of age, followed by a more or less variable period up to 4 months, when there was a gradual rise to 6 months of age. The average inorganic blood phosphorus content for all calves used, from 0 to 185 days old, was 7.26 mgm. of phosphorus per 100 cc. of plasma.

After 6 months, there began a slow, gradual decline in the blood phosphate. Calves at an average age of 274 days had a

TABLE 8

*Effect of age on the inorganic phosphorus content of cattle blood*

| NUMBER OF ANIMALS | RANGE OF AGES | AVERAGE AGE | AVERAGE INORGANIC P IN 100 CC. |
|---|---|---|---|
| | *days* | *days* | *mgm.* |
| 5 | 0 to 7 | 3 | 5.97 |
| 6 | 25 to 35 | 29 | 7.03 |
| 6 | 55 to 65 | 59 | 7.57 |
| 6 | 85 to 95 | 91 | 7.19 |
| 7 | 115 to 125 | 121 | 7.53 |
| 7 | 145 to 155 | 149 | 7.68 |
| 9 | 175 to 185 | 180 | 7.89 |
| Average..... | 0 to 185 | 90 | 7.26 |
| 5 | 269 to 280 | 274 | 7.25 |
| 6 | 306 to 365 | 337 | 7.20 |
| 5 | 406 to 463 | 436 | 7.02 |
| 4 | 497 to 547 | 527 | 5.91 |
| 3 | 559 to 589 | 575 | 5.55 |
| 3 | 600 to 650 | 626 | 5.94 |
| *Average..... | Mature cattle | | 5.87 |

* The average given is one obtained by Robinson and Huffman (15).

mean inorganic phosphorus content of 7.25 mgm. per 100 cc. of plasma. At an average age of 436 days, the value had decreased to 7.02 mgm., and at 527 days there was an average blood phosphate content of 5.91 mgm. per 100 cc. of plasma. After this age the results were variable but with no pronounced change up to 626 days. At this time they had apparently reached approximately the normal value for mature cattle.

## DISCUSSION OF RESULTS

The inorganic phosphate in cattle blood cannot be determined with accuracy by random sampling. It appears that wide fluctuations may occur from day to day in consecutive three-day tests even when the samples are taken under presumably identical physiological conditions. Although calculation from 60 such three-day series shows a very high coefficient of correlation between the consecutive samples, yet nearly 50 per cent of the individual samples varied 10 per cent or more from the regression line, a few showing over 100 per cent variation from the expected value. Of the 3 physiological factors studied, namely, exercise, food ingestion, and water drinking, only the first gave results of sufficient significance to account for the abnormally great fluctuations. Our study seems to show fairly definitely that vigorous exercise usually causes an increase in the inorganic blood phosphorus, followed within a half-hour by a marked decrease to a point below the "before exercise" level. The blood phosphate usually remains low for a period of at least 2 hours after exercise. The amount of increase observed in these trials seemed to depend on the severity of the exercise and upon getting the blood sample quickly after exercise ceased. It would appear that the increase is more or less transitory, but that the decrease which follows persists for several hours. The increase in inorganic blood phosphorus as a result of exercise is possibly caused by a breakdown in hexose phosphates, to supply energy, thereby releasing inorganic phosphorus into the blood. The decrease which follows is, by analogy, the result of a resynthesizing of hexose phosphates. It would seem that any severe resistance of an animal to the act of bleeding would cause changes in the inorganic phosphorus of the blood, but that in animals that submit meekly to this process, there would be no significant change.

A significant but small increase in blood phosphate appears to occur within the first hour of the normal food ingestion in most cattle. This may be great enough to account for day-to-day fluctuations of 5 to 10 per cent from the expected value in case no attempt is made to control the blood sampling with reference

to periods of time in close proximity to the normal period of feeding. In general, this rise seems to persist for about 2 hours after the food ingestion, which is followed within the next hour by a fall which brings the blood phosphate to approximately the original value before feeding. It would appear that bleeding time should be either before feeding or about three hours after feeding, if the effect of food ingestion on blood phosphate is to be avoided. It is doubtful whether food ingestion caused any of the day-to-day fluctuations in our observations, because the blood samples were all taken at the same hour each day, thus bringing them to the same proximity to the time of feeding.

The inorganic blood phosphate in cattle does not respond to food ingestion in the same manner as is produced by the ingestion of soluble carbohydrates by man and small animals. The depression noted by others to occur immediately after glucose ingestion does not occur in cattle until about three hours after feeding. Glucose from digested carbohydrates evidently does not reach its height of absorption until about three hours after food ingestion and even then the effects on inorganic blood phosphate through the formation of hexose phosphates are mild. The increases in inorganic phosphate occurring soon after eating may be due primarily to the release of phosphate from the tissues as a result of muscular activity accompanying the peristalsis of the extensive digestive system of this species.

The effect of water drinking on inorganic phosphate in cattle blood is negligible. The fluctuations noted were under 5 per cent even when the cattle were deprived of water as long as 44 hours. Under normal conditions one cannot attribute day-to-day fluctuations to this factor.

A marked decrease in the inorganic phosphorus content of cow's blood occurs at or near the time of parturition. The amount of decrease noted in this study varied from 1.3 to 3.2 mgm. of inorganic phosphorus per 100 cc. of plasma. The decrease sets in on the day before calving and usually persists for several days. The lowest point of decrease may occur either before or after parturition but usually within 2 days of that event. The reason for this marked decrease in blood phosphate at the time of par-

turition is not fully understood, but it is probably associated with some special metabolism taking place at that time. Since we know that phosphorus is associated with the metabolism of both fat and glucose, one would suspect that the rather sudden disappearance of much of the phosphates from the blood as a result of calving is associated with some rapid metabolism or storage of these compounds.

The inorganic phosphorus content of calf blood is much higher at birth than that of the dam. The level of phosphate content in calf blood at birth compares favorably with that of the dam's blood several days before calving. Evidently, the fetus is influenced in its blood phosphate content by the dam, but when the dam decreases in blood phosphorus at or before calving, the calf maintains a higher level. In the cases observed, the calves showed a marked increase in inorganic blood phosphorus content within 2 days after birth, showing that when on an independent diet they can quickly build up the blood phosphorus level.

During the first week after birth calves are lower in inorganic blood phosphorus than older calves, but higher than their dams. In the cases studied, they increased to a maximum at about 180 days of age. The average inorganic phosphorus content of blood at this age was 7.89 mgm. per 100 cc. of plasma. The average content of inorganic phosphorus in the blood of calves 0 to 185 days old was 7.26 mgm. per 100 cc. of plasma. After an average of about 180 days, the trend of blood phosphate seems to be downward. The number of animals used in this study was too small to make the results conclusive, but they indicate that calves ordinarily increase in blood phosphate until about six months of age, then gradually decrease until they reach the normal for mature cattle at about eighteen months old.

## CONCLUSIONS

The inorganic blood phosphate in individual cattle may vary markedly from day to day even when the blood is drawn under apparently identical conditions.

The inorganic blood phosphate in individual cattle may vary

considerably from hour to hour, although the data available on this point are very limited.

Exercise causes marked changes in blood phosphate of cattle. There is first a definite rise, which is followed by a marked fall which persists for several hours.

Feeding has a small but significant effect on the inorganic phosphate in cattle blood. The value rises within the first hour and apparently does not return to normal until after about three hours.

Normal water drinking by cattle has no significant effect on blood phosphate.

So far as the factors studied are concerned, the procedure indicated for securing normal samples of blood from cattle are to have the animal at rest in its stanchion for several hours and draw the blood before feeding, with the least possible physical disturbance of the animal. Water should be allowed *ad libitum*.

Parturition causes a decrease in the inorganic blood phosphorus which may amount to as much as 3.2 mgm. per 100 cc. of plasma. The decrease sets in on the day before calving, the lowest point of decrease occurring either before or after parturition.

The phosphate content of calf blood at birth is higher than that of the dam, and compares favorably with the value shown by the dam several days before calving.

Calves increase in the inorganic phosphorus content of their blood until about six months old, after which a decrease sets in which continues until the normal range for mature cattle is attained.

The blood analyses shown in tables 1 and 2 were made by W.M. Neal, Special Analyst for the mineral nutrition project of the Experiment Station. Credit is due Professor J. Arthur Harris, Station Biometrician, for the formulae used in connection with the day-to-day variations, and to Miss Rachel Rude, Station Assistant, for the calculations.

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# A STUDY OF THE RELATION BETWEEN THE TIME A COW IS CARRIED IN UTERO AND HER MATURE EQUIVALENT BUTTERFAT PRODUCTION*

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This study was originally suggested by Dr. W. L. Williams, Emeritus Professor of Cornell University, in personal correspondence to the authors in which he raised the question of the possibility of the producing efficiency of a cow being influenced by the duration of time she was carried in utero by her dam. He goes on further to say,

> first you will perhaps regard this as absurd, but in thoroughbred horses, V. Ottingen, stud-master at Trakehnen, one of the most famous imperial studs in Europe, has declared that a foal carried over 335 days in utero rarely distinguishes itself in later years and that a foal carried over 345 days virtually never proves satisfactory. He submits considerable important evidence in support of his contention.
>
> Prolonged gestation in the mare somewhat paradoxically appears to be the analogue of abbreviated pregnancy in cows and referable to identical or allied causes. Therefore, the question has arisen in my mind, is there a most favorable period of gestation for dairy cattle.

It is a common occurrence for living calves to be born several days or even weeks before the end of the normal gestation period and it is of economic importance to know whether this abnormal physiological condition affects the productive ability of the animal.

In connection with this study a second problem was considered, that is, the relation between the age of the cow and the length of time she carries her fetus in utero.

## REVIEW OF LITERATURE

Hammond (2) in summarizing the opinion of practical breeders of beef cattle, states there is no difference in the length of gesta-

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tion of heifers and mature cows. He gives the average gestation for heifer calves as 38 weeks and for male calves as 41 weeks.

Wright (5) gives the average length of gestation for the cow as 280 days.

Wing (4) reports from the result of observations on one herd over a period of 10 years that the average gestation for 182 births was 280 days. The shortest period was 264 days and the longest 296 days. There were equal numbers for each day from 274 to 287 days. There was no difference in the average observed for males and females. Many cows showed well marked individual characteristics as to periods of gestation which may be several days longer or shorter than the average.

A summary of the breeding records of the Clemson Agricultural College herd (1), including Jerseys, Guernseys and Holsteins, shows an average length of gestation of 279.12 days for 194 females and 280.26 days for 192 males dropped in this herd. When the gestation period for animals at different ages, ranging from 2 to 14 years, was determined it was evident that no positive relation existed between the age of the cow and the length of her gestation period.

## SOURCE OF DATA

Animals of the Jersey breed were chosen for study. Two groups were selected. One group of 406 cows selected from the 1926 and the 1927 supplements of the Register of Merit included only animals which had 365 day records made in AA class and which were milked three times per day or 1095 times during the year. The second group of 340 cows selected from the 1926, 1927, and 1928 supplements of the Register of Merit contained only animals with 365 day records made in AA class and milked twice per day or 730 times during the year.

Through the courtesy of Mr. L. W. Morley, Secretary of the American Jersey Cattle Club, the dates of service of the dams of these cows were secured. The dates on which these cows were dropped and also the dates on which their dams were dropped were secured from the volumes of the Jersey Herd Register.

In order to secure comparative values from records studied, all

TABLE 1

*Correlation surface for butterfat production of Jersey cows milked three times per day and the length of time they were carried in utero by their dams*

| CLASS—COWS' YEARLY BUTTERFAT PRODUCTION | LENGTH OF GESTATION PERIOD IN DAYS | | | | | | | | | | | | | FREQUENCY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 253 | 256 | 259 | 262 | 265 | 268 | 271 | 274 | 277 | 280 | 283 | 286 | 289 | 292 | |
| *pounds* | | | | | | | | | | | | | | | |
| 400–449 | | | | | | | 1 | 2 | 1 | 1 | 3 | 1 | | | 9 |
| 450–499 | | | | 1 | | 1 | 1 | 2 | 6 | 4 | 6 | 1 | 1 | | 23 |
| 500–549 | | | | | | 1 | 3 | 4 | 4 | 8 | 3 | 1 | 1 | | 25 |
| 550–599 | | | | | | | 1 | 7 | 10 | 18 | 12 | 2 | 2 | | 52 |
| 600–649 | 1 | | 1 | | 1 | | 3 | 8 | 16 | 22 | 16 | 7 | 2 | 1 | 78 |
| 650–699 | | | | | | | 4 | 10 | 11 | 24 | 11 | 3 | 1 | 1 | 65 |
| 700–749 | | | | | | 1 | 5 | 5 | 12 | 17 | 10 | 8 | 2 | | 30 |
| 750–799 | | | | 1 | 1 | 2 | 3 | 1 | 11 | 14 | 7 | 2 | 1 | | 43 |
| 800–849 | | | | | | | | 3 | 5 | 5 | 6 | 2 | | 1 | 22 |
| 850–899 | | | | | | | | 4 | 5 | 3 | 2 | 3 | | | 17 |
| 900–949 | | | | | | | 1 | 2 | 1 | | 1 | | | | 5 |
| 950–999 | | | | | | | 1 | 1 | | | 2 | | | | 4 |
| 1000–1049 | | | | | | | | | | 1 | 1 | | | | 2 |
| 1050–1099 | | | | | | | | | | | 1 | | | | 1 |
| Frequency.. | 1 | 0 | 1 | 2 | 2 | 5 | 23 | 49 | 82 | 117 | 81 | 30 | 10 | 3 | 406 |

TABLE 2

*Correlation surface for butterfat production of Jersey cows milked twice per day and the length of time they were carried in utero by their dams*

| CLASS—COWS' YEARLY BUTTERFAT PRODUCTION | LENGTH OF GESTATION PERIOD IN DAYS | | | | | | | | | | | | | FREQUENCY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 256 | 259 | 262 | 265 | 268 | 271 | 274 | 277 | 280 | 283 | 286 | 289 | 292 | |
| *pounds* | | | | | | | | | | | | | | |
| 400–449 | 1 | | | | 1 | 1 | 4 | 2 | 15 | 7 | 5 | | | 36 |
| 450–499 | | 1 | | 2 | 1 | 3 | 13 | 17 | 29 | 21 | 2 | 3 | | 92 |
| 500–549 | | | 1 | | 1 | 6 | 5 | 13 | 22 | 13 | 3 | 2 | | 66 |
| 550–599 | | | | | 3 | 4 | 11 | 15 | 11 | 14 | 3 | 2 | 1 | 64 |
| 600–649 | | | | | 1 | 2 | 3 | 11 | 12 | 9 | | | | 38 |
| 650–699 | | | | | | | 3 | 5 | 11 | 4 | | 1 | | 24 |
| 700–749 | | 1 | | | 1 | 1 | | 2 | 2 | 2 | | 1 | | 10 |
| 750–799 | | | | | | | | 1 | 3 | 1 | 2 | | | 7 |
| 800–849 | | | | | | | | | | 1 | | | | 1 |
| 850–899 | | | | | | | | 1 | | | | | | 1 |
| 900–949 | | | | | | | | | | | | | | 0 |
| 950–999 | | | | | | | | | 1 | | | | | 1 |
| 1000–1049 | | | | | | | | | | | | | | |
| Frequency.. | 1 | 2 | 1 | 2 | 8 | 17 | 39 | 67 | 106 | 72 | 15 | 9 | 1 | 340 |

records were converted to a mature equivalent basis by multiplying the yearly fat record by the different age conversion factors as used by Turner (3).

The term fat record hereafter in this discussion will refer to a mature or mature equivalent record. In case an animal had more than one Register of Merit record the highest mature equivalent record was used.

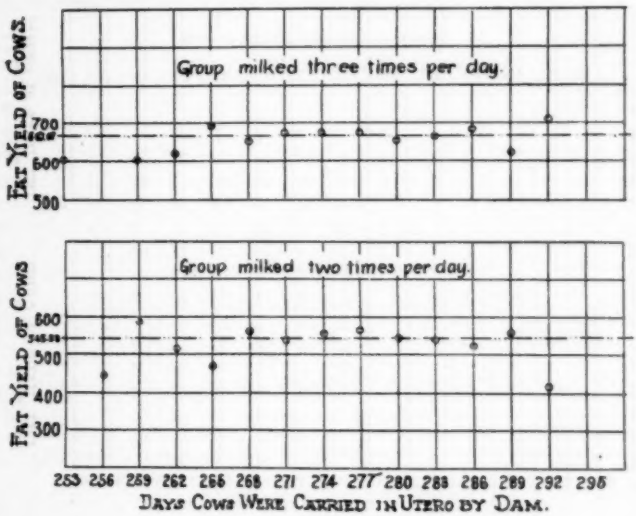


Fig. 1. Average Butterfat Production of Cows Carried Different Lengths of Time in Utero

## PRESENTATION OF DATA AND DISCUSSION OF RESULTS

In the first study the mature equivalent butterfat records of the two groups of cows were grouped according to the length of time each cow was carried in dam as shown in the correlation surfaces (tables 1 and 2).

The coefficient of correlation for the data presented in table 1 is 0.0135 $\pm$0.0335 and for that in table 2 is 0.0076 $\pm$0.0366.

The arithmetical averages of production for the different length

of gestation groups were determined and are presented graphically in figure 1.

In each case the coefficient of correlation is smaller than the probable error. Also, when the averages are plotted it will be noted that each group falls very close to the mean for the entire lot.

TABLE 3

*Correlation surface for age of Jersey cows and the length of their gestation periods*

| CLASS—AGE OF COWS | LENGTH OF GESTATION PERIOD IN DAYS | | | | | | | | | | | | | | FREQUENCY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 253 | 256 | 259 | 262 | 265 | 268 | 271 | 274 | 277 | 280 | 283 | 286 | 289 | 292 | |
| *years* | | | | | | | | | | | | | | | |
| 1 | | | | | | 3 | 2 | 1 | 7 | 8 | 7 | 1 | | | 29 |
| 2 | | 1 | 1 | 1 | 2 | 4 | 5 | 13 | 29 | 30 | 23 | 3 | 2 | | 114 |
| 3 | | | | | | 2 | 4 | 22 | 24 | 47 | 24 | 12 | 5 | | 140 |
| 4 | | | | | | | 6 | 20 | 15 | 19 | 16 | 5 | 2 | | 83 |
| 5 | | | | | | 1 | 5 | 5 | 14 | 28 | 15 | 7 | 4 | | 79 |
| 6 | 1 | | | 1 | | 3 | 6 | 8 | 13 | 27 | 14 | 6 | 2 | 1 | 82 |
| 7 | | | | | | | 2 | 3 | 9 | 19 | 15 | | | 1 | 49 |
| 8 | | | 1 | 1 | | | 2 | 2 | 11 | 12 | 10 | 4 | 2 | | 45 |
| 9 | | | 1 | | 2 | | 2 | 2 | 6 | 12 | 8 | | | | 33 |
| 10 | | | | | | | 2 | 1 | 8 | 6 | 4 | 1 | | | 22 |
| 11 | | | | | | | 2 | 1 | 6 | 4 | 2 | 1 | | 2 | 18 |
| 12 | | | | | | | 1 | 1 | 3 | 1 | 3 | 2 | | | 11 |
| 13 | | | | | | | | | 3 | 1 | 1 | | | | 5 |
| 14 | | | | | | | | 1 | 1 | 2 | 2 | 1 | | | 7 |
| 15 | | | | | | | | | 1 | 1 | 3 | | 1 | | 6 |
| 16 | | | | | | | | 1 | | 1 | | | | | 2 |
| 17 | | | | | | | | | | | | | | | 0 |
| 18 | | | | | | | | 1 | | | | | | | 1 |
| Frequency. | 1 | 1 | 3 | 3 | 4 | 13 | 39 | 82 | 150 | 218 | 147 | 43 | 18 | 4 | 726 |

According to these data there is no indication that any particular length of fetal development has a significant effect on the butterfat production of the cow if this gestation period is between 253 and 293 days.

In the second part of this study the length of the gestation periods were grouped according to the age of the cows as indicated in the correlation surface (table 3).

The coefficient of correlation for the data presented in table 3 is 0.0551 ±0.0249.

The average lengths of gestation for the different age groups are presented graphically in figure 2.

The coefficient of correlation and the small degree of variation of the averages of the different age groups from the mean length of gestation for the entire group indicates that the length of gestation is not influenced by the age of the cow.

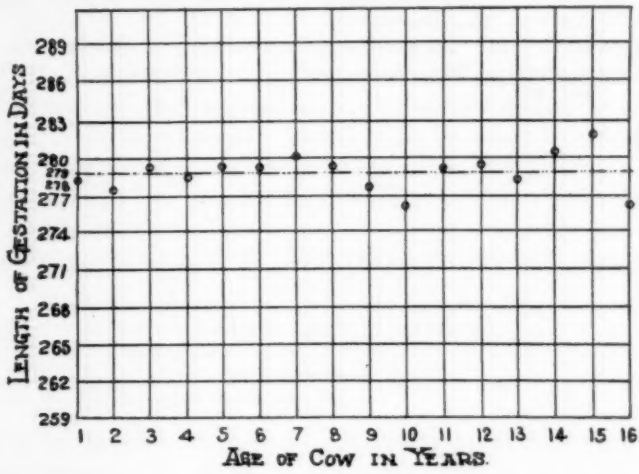


Fig. 2. Average Length of Gestation of Jersey Cows at Different Ages

Attention is called particularly to the average length of gestation of all groups, including 726 animals, which is 278.9 days, a somewhat shorter period than is generally accepted as the normal gestation period for Jersey cows.

## SUMMARY

1. These data indicate that the mature equivalent butterfat records of Jersey cows are not influenced by the length of time these cows are carried in utero by their dams.

2. The length of time these cows were carried in utero was not influenced by the age of their dams.

3. The average length of the gestation period for 726 Jersey cows was 278.9 days.

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# SOME OBSERVATIONS ON PROCESSED CHEESE*

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Processed cheese is a comparatively recent product, but it has taken a very important place in the cheese industry, a high percentage of the total cheese output being marketed in this form. The advantages of this form of cheese have been very clearly stated by Davel and Retief (1).

In its manufacture there are various factors that affect the flavor, appearance and physical properties of the finished product. The only knowledge available on these factors is such information as has been accumulated in practical work. It is the object of this paper to give a report on these factors from a more scientific standpoint.

In the experimental work the following factors were studied:

1. The addition of the various so-called emulsifiers
2. The temperature treatment in processing
3. The moisture content
4. The reaction of the cheese
5. The age of the cheese used

To note the effect of these factors, observations were made on the behavior of the cheese in processing and on the following properties of the finished product:

1. The moisture content
2. The reaction, both pH and titratable acidity
3. The body of the cheese
4. Slicing properties
5. Color
6. Keeping quality
7. Appearance of the tinfoil

In addition to the above, there have also been some observations made on the processability of frozen cheese and the distribu-

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tion of nitrogen between the water and 5 per cent salt soluble forms in the cheese before and after processing.

The kettle used, figure 1, was made in the University shops from plans drawn by us after the general design of kettles used commercially. When filled to capacity, it could handle about sixteen pounds of cheese, but for the experimental work it was decided to use eight pounds as this was enough to cover the bulb of the thermometer and could be easily handled. To empty the kettle the entire stirring device was raised without stopping its action and this in turn raised a plug in the bottom of the kettle that permitted the molten cheese to flow out.

In order to have a record of the temperature of the cheese in the kettle, a special thermometer was devised, the bulb of which formed part of the stirrers. This was connected by means of a fine copper tube to a glass capillary tube. The entire system revolved with the stirrers and the temperature was read on a stationary scale mounted in back of the capillary. The liquid used in this thermometer was xylene colored with Sudan III. With this device, it was possible to note the temperatures at which changes took place in the cheese during the heating.

As the body of the cheese is a criterion of its quality, it was thought advisable to have some means of determining the body in which the personal factor would be eliminated as far as possible. This was accomplished by means of the instrument shown in figure 2, which was designed especially for this purpose by the authors. The body of the cheese was measured by determining the force required to crush an inch cube to one-half of its original thickness. The force was applied by allowing mercury to flow into a beaker, placed as shown, and the stream of mercury was automatically shut off by an electrical device when the cheese had been crushed to 0.5 inch in thickness. The results are reported throughout in terms of the weight of the mercury and the beaker. To give the actual force applied, the figures must be multiplied by 5 since the mechanical advantage of the pulleys shown is 5. The crushing strength of cubes from the same sample of cheese differed appreciably due to the unavoidable variations

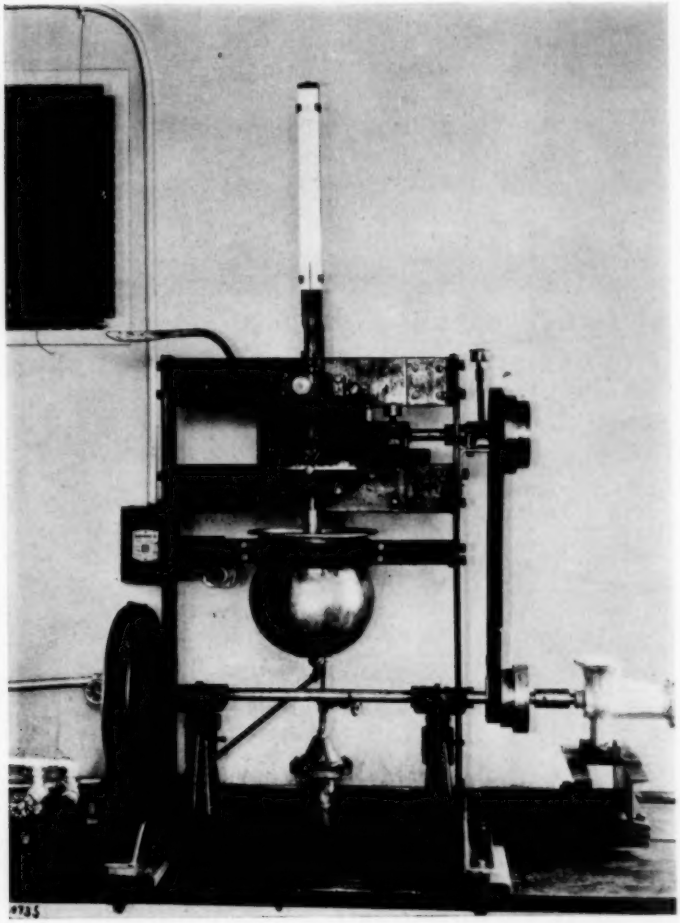

FIG. 1. EXPERIMENTAL CHEESE PROCESSING KETTLE

within the cheese. For this reason from 6 to 10 measurements were made on each sample and the results reported here are averages as too much space would be required to present all the data that have been collected.

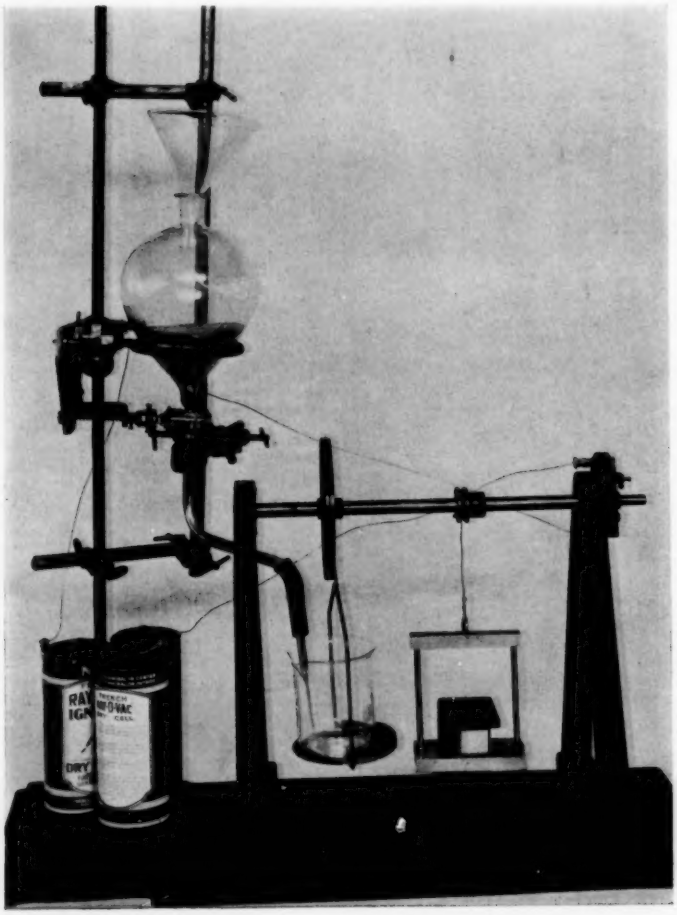

FIG. 2. DEVICE FOR MEASURING THE BODY OF CHEESE

## EXPERIMENTAL

With this description of the apparatus used, let us now turn our attention to the salts commonly added to cheese, in addition to the common salt (NaCl) of which about one-half of one per

cent is used. For want of a better term, these salts are commonly called emulsifiers. Three have been used quite extensively either alone or in mixtures. They are sodium citrate, di-sodium phosphate containing traces of the tri-sodium salt, and sodium potassium tartrate or Rochelle salts. These salts are added for the purpose of preventing fat separation and controlling the body and texture of the cheese during and after processing. The separation of the fat may be affected by the age of the cheese used, the reaction, moisture content, and the rate of stirring. It is possible to select cheese in which these factors are combined in such a balance that no fat separation will occur at any time, and again the fat may separate as the heating is started and be reincorporated as the temperature approaches 130°F. In such cases the use of these salts is unnecessary in the prevention of the fat separation, but even in these cases their addition may be decidedly beneficial on the body and texture of the finished product.

At present there are no tests that can be used to give an indication of the processability of cheese at the time of its selection except the use of a small experimental kettle. As cheese is chosen on the basis of quality, especially flavor, and not with reference to its action during processing, the use of salts that have a controlling effect on fat separation and on body and texture is of importance to the commercial operator.

At the present time the use of Rochelle salts is on the decrease, due possibly to the fact that gritty, "sandy" particles are sometimes found in the cheese. These crystals have been identified by us as calcium tartrate. A series of experiments using Rochelle salts as the emulsifying agent failed to show any remarkable results in the processed cheese. Besides the presence of calcium and tartrates in the cheese, there seem to be other undetermined factors involved in the formation of the gritty calcium tartrate crystals.

The curves shown in figure 3, give a comparison of the effect of di-sodium phosphate and sodium citrate, used separately and in mixtures, on the body of cheese. It will be noted in each case that the citrate gives the firmer body and that the body decreases with the increase of phosphate in the mixture. It is impossible to give

a very definite figure for the best body, but after a consideration of the results of tests made on experimental and commercial samples of cheese, it would seem that an inch cube of cheese requiring a crushing force of 800 to 1200 grams with a mechanical

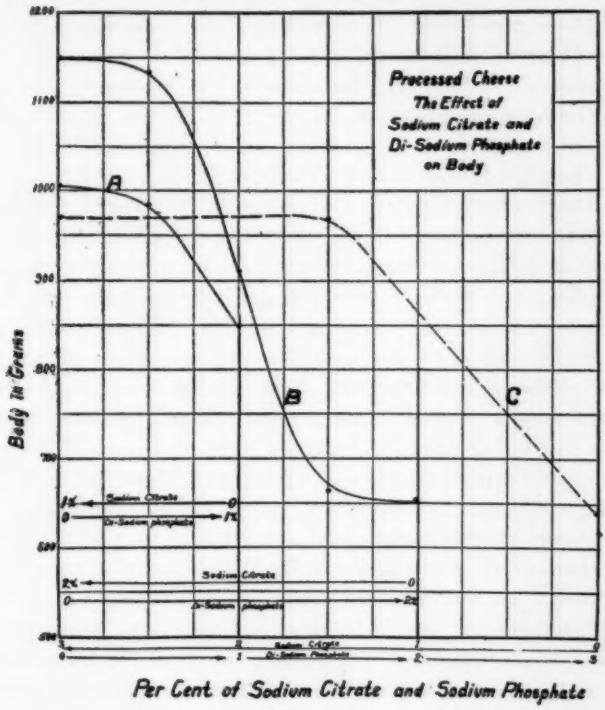


FIG. 3

The total amounts of the two emulsifiers, used alone and in combination, in graphs A, B, and C were always 1, 2, and 3 per cents, respectively.

advantage of 5, should be considered as having a desirable body. The citrated cheese in each case falls within these limits.

When the amount of di-sodium phosphate is increased beyond the limits shown in the curves, the body becomes more firm and then as the amount of the salt is increased, this is succeeded by a

weaker body accompanied with fat separation and grainy texture. With sodium citrate the addition of more of the salt did not cause any very marked changes in the body of the cheese and the use of 9.8 per cent sodium citrate did not cause fat separation. For commercial cheese-making the use of more than 2 per cent of these salts, especially the phosphate, should be avoided as it tends to give the cheese a bitter flavor when used in larger proportions, and the tin foil is darkened. Citrate on the other hand showed no evidence of darkening the tin foil except when very large amounts were used or when some alkaline salt such as sodium carbonate was added. When the reaction of the cheese, in terms of pH, was above 6.3, the tin foil would be discolored, and the darkening increased as the reaction approached neutrality. Unless it is stated otherwise, sodium citrate was used as the emulsifier in all the experimental cheese made.

Another interesting fact was brought out when cheese mites invaded the room in which the cheese was stored. They rapidly attacked the cheese in which citrate was used, whereas the cheese in adjacent boxes that was emulsified with phosphate was either untouched or only slightly eaten. The action of the cheese mites tended to give the cheese a more alkaline reaction. While this action of the cheese mites may be taken as indicative of a preservative action of the phosphate, the manner in which the citrated cheese was consumed would seem to be evidence that the mites preferred it to the other.

A comparison of two forms of sodium citrate, namely, $2Na_3C_6H_5O_7\ 11H_2O$ (U.S.P. VIII) and $Na_3C_6H_5O_7 2H_2O$ (U.S.P. X) failed to show any differences in the action of the two salts.

A study of the temperature to be used in the processing of cheese was made from the standpoint of the degree that must be reached and also the length of time that the cheese may be held at the temperature. From the curve shown in figure 4, it is evident that there is little change in the body of the cheese when it is drawn from the kettle as soon as possible after temperatures of 140° to 150°F. had been reached. In cheese processed below 140°F. there was a formation of gas during storage indicating that these temperatures were insufficient to inhibit the growth of

bacteria. The odor of the cheese gave further evidence of putrefactive changes. With the higher temperatures the body of the cheese increased rapidly. In attaining these temperatures, there is, of course, greater opportunity of evaporation and this in part may account for the firmer body, but there are undoubtedly other factors. With the higher temperatures, it was noted that the color changed to a "salmon pink," the intensity increasing the

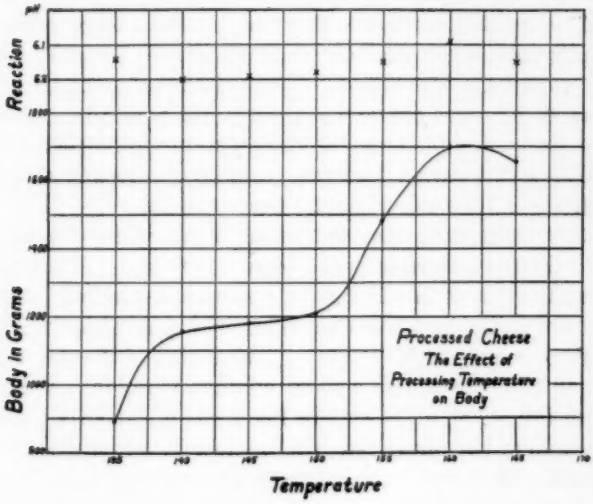


FIG. 4

temperature. This change of color confirms the observations of Davel and Retief (1).

When the cheese was held at 140° to 155°F. for 3 to 10 minutes after reaching the desired temperature, the increase in body was almost directly proportional to the holding time. There was very little change in color at these temperatures, but holding for any length of time at the higher point caused a distinct color change.

In considering these temperatures it must be remembered that the thermometer bulb was in the center of the mass of cheese and

was revolving with the stirrers at all times. With the type of stirrer used and the speed at which they revolved, it was safe to assume that all the cheese was heated to the temperature recorded by the thermometer.

For all experimental work except when otherwise indicated the cheese was drawn from the kettle as soon as possible after the temperature had reached 148° to 150°F.

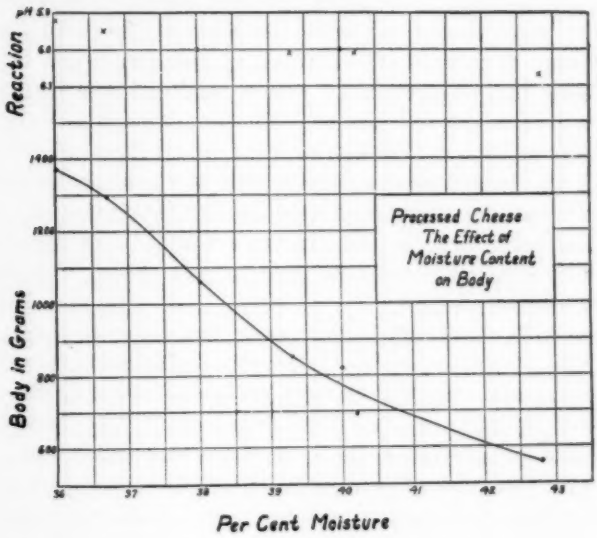


FIG. 5

The relation between the moisture content and the body of the cheese is shown by the curve in figure 5. This indicates that as long as all other factors are kept relatively constant the body of the cheese will decrease with the increase in moisture. It will be noticed that the figures previously given for a desirable body correspond with moisture contents of 38 to 40 per cent. There seems to be a rather close relationship between the amount of water in a cheese and the way in which it can be cut with a commercial meat slicer that has been set to cut a section 0.02 inch in thickness. If

the cheese contains from 37 to 40 per cent moisture, it will be cut quite easily and give a very uniform slice. Below this the cheese is rather brittle and breaks as it is cut, while cheese containing excess water tends to stick to the revolving knife, and will not cut evenly except when the knife has been set to cut a thicker slice.

Studies on the loss of moisture during processing indicate that there are a number of factors that must be considered. If the kettle is of the wide and rather flat type, it will be necessary to use more water than if the kettle has more nearly vertical sides. The amount of cheese in the kettle is of importance also as there is a greater loss proportionally with a small amount than if the kettle is filled nearly to capacity. The actual loss may vary from practically none to as high as 3 per cent, and if the time necessary to reach the desired temperature is long, the loss may go as high as 5 per cent. In commercial work the man operating the kettle will, after some practice, be able to estimate very closely the amount of water that must be used to give cheese with the required moisture content.

In the work described in this paper, the water of crystallization of the emulsifiers was not taken into consideration as in most cases it was less than the amount necessary to bring the water content of the salts added up to the 40 per cent limit. When other factors were being studied, the amount of water added was sufficient to give the finished product 38 to 39 per cent moisture. In the experimental cheese where rather large amounts of phosphate were used (approximately 50 per cent water) the moisture content of the finished product was somewhat higher than 39 per cent).

It is possible to have wide variations in the body of the cheese and still keep the amount of moisture present nearly constant. A most striking example of this will be pointed out on another curve in which two adjacent points have a difference of 2000 grams in body and only 0.2 per cent difference in moisture with the cheese having the higher moisture also having the higher body. If an excess of water is added during the processing, the body of the cheese is weakened, there is a tendency for the fat to separate in spite of emulsifiers, and the finished product may be best described as coarse and grainy.

When the relation between the body and the reaction is considered, the very interesting curve shown in figure 6 is obtained.

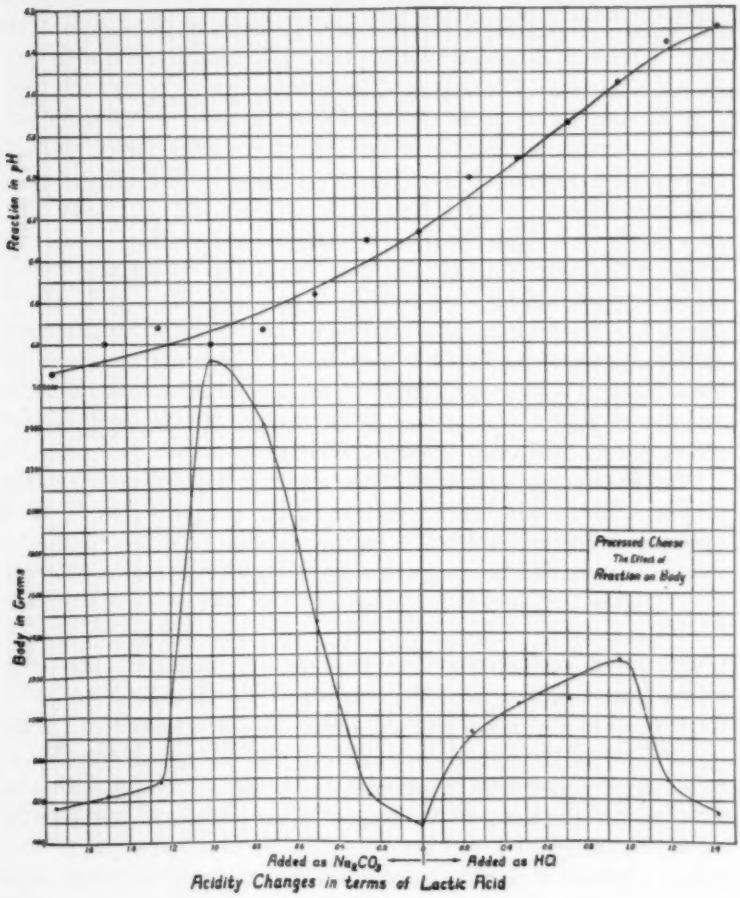


FIG. 6

In this series of experiments the amount of emulsifier used was kept constant and the other reagents added in the amounts indicated. The increase in the body with the addition of alkali is

very remarkable and the sudden break beyond 1 per cent is rather unexpected. It is at this break that we find the previously mentioned difference in body with the moisture content remaining the same. If one plots the reaction in terms of pH against the body, a curve is obtained that is practically identical with the one shown. As was to be expected from the work on the emulsifiers and the use of phosphate, the tin foil of the more alkaline cheese was very dark and this color was imparted to the cheese so that there was a discolored layer on the outside of the cheese that was in some cases nearly half an inch deep. This cheese was also gassy while the cheese at the more acid reaction was not, which would indicate that the reaction of the cheese may influence the pasteurizing temperature to be used. The acid side of the curve shows an increase in body, that is not as pronounced as the other and the break is less abrupt. It is not necessary to use a mineral acid to get a curve of the nature shown as a similar one was obtained when a part of the sodium citrate used was replaced by an equivalent amount of citric acid.

The reaction curve shown in the same figure indicates a fairly uniform change with each addition of the reagents and is more uniform on the acid side than on the alkaline. This is no doubt due to the combination of the reagents with the casein and fat of the cheese and is best illustrated by the texture of the cheese. With the more acid cheese the body was coarse and grainy giving a very rough surface when cut showing fine particles, there was also a greasy film around the cheese. With the alkaline cheese there was less graininess and a tendency towards soapiness. The layer of fat on the outside was deeper and the taste of the cheese was very disagreeable being very bitter as compared with the sour taste of the more acid cheese. When citric acid was used in small amounts (0.1 to 0.2 per cent) the taste of the product was rather pleasant giving the impression that the original cheese was somewhat older than was actually the case, but if the amount of acid was increased, the sour flavor was more pronounced making the cheese rather unpleasant to the taste.

It is impossible to show any very definite relationship between the titratable acidity of the cheese as expressed in per cent of lac-

tic acid and the body. The titratable acidity in connection with the reaction in terms of pH may be used to give an idea of the kind of cheese and the emulsifier that was added when examining

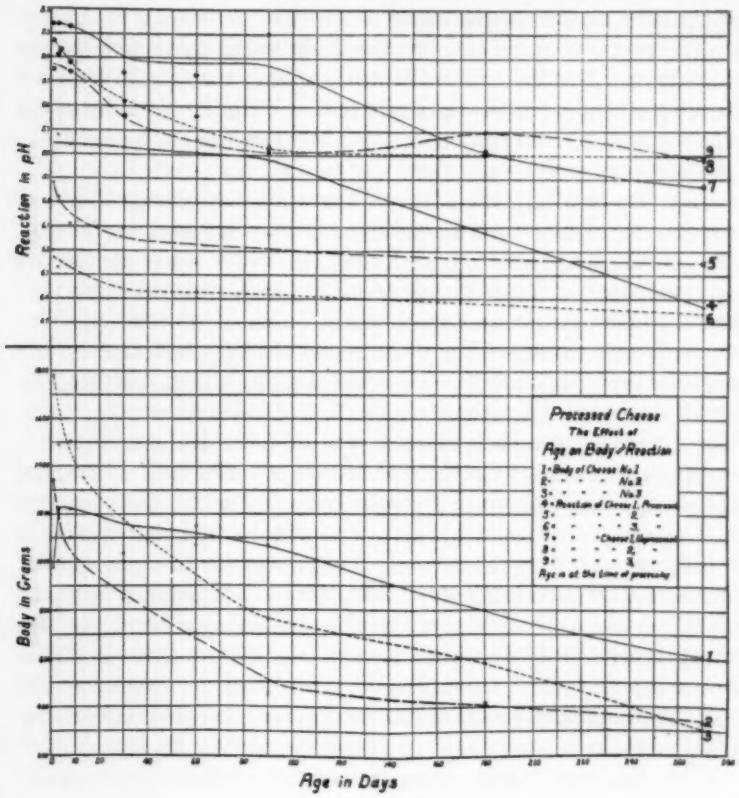


FIG. 7

cheese of unknown processing history. A high value for the titratable acidity coupled with a low pH (5.8 or lower) would indicate that either a very acid cheese had been used or that acid had been added during the processing. If, however, the titrat-

able acidity is high and the pH high (6.4 or higher) this could be considered as evidence of the use of an excessive amount of emulsifier especially phosphate. A low titratable acidity with a high pH would be indicative of the addition of free alkali at some time during the making of the cheese.

The reaction of typical processed cheese in terms of pH may vary from 5.8 to 6.3. The titratable acidity may range from 1.1 to 1.8 per cent calculated as lactic acid. With the blending of the cheese as it is done commercially, the reactions of the resultant product stay well within these limits.

In order to study the effect of age upon the processability of cheese, experiments were conducted with three series of cheese. The cheese was made up as Young Americas (each containing approximately ten pounds) so that a single cheese would furnish material enough for an experimental batch in processing. One cheese from each series was processed at the ages indicated on the curves shown in figure 7. Two had sodium citrate used as the emulsifier while the other, cheese 3, had di-sodium phosphate. The curves for the body show a very uniform decrease with the exception of cheese 1 for the first few days. Cheese 3 containing the phosphate had a much stronger body at first but the decrease with age was more rapid and after 9 months we find that the body is much poorer than the others. The curves for the reaction before and after processing show that there is a change of approximately 0.4 of a pH unit towards the alkaline side when sodium citrate is used, while di-sodium phosphate shows an average difference of about 0.6. Cheeses 2 and 3 were made on successive days and the parallelism between the reaction curves after processing is very striking.

With the very young cheese it was impossible to process it without the separation of fat even with the use of emulsifiers and the fat was not re-incorporated as the temperature increased. After the cheese was 8 days old, this difficulty was not encountered again with any of these series. With cheese more than a year old, this difficulty may occur again, but it can be overcome to a large extent by increasing the amount of the emulsifier. The cheese at the age of 9 months tended toward a very grainy texture

and the use of phosphate seemed to make this defect more pronounced. With the young cheese, the body was very firm and the cheese as it came from the kettle did not flow together but formed rather distinct layers. Considerable air was held in such cheese in pockets of various sizes making it rather difficult to secure inch cubes that were free from these imperfections. These pockets often contained free butterfat that had been carried along as the cheese was run out of the kettle. The flavor of this young cheese was the same as the unprocessed green curd and the texture was rather rubbery.

In the commercial manufacture of processed cheese, many of the difficulties that have been mentioned here as due to the age and reaction of the cheese used may be overcome by blending. This consists of the selection of cheese of such ages and degree of ripeness that the product will be free from all the defects that have been mentioned.

This is usually accomplished by mixing two or three parts of relatively young cheese, about two months old, with one part of older cheese, depending upon the flavor that is desired in the product. If the cheese has been properly chosen and the average age is about four to seven months, it is reasonable to expect a very satisfactory product.

In connection with a study on the freezing point of cheese (2), a series of experiments were conducted in order to determine whether freezing affected the processability of the cheese in any way. There were 6 samples of cheese of different ages and in various stages of curing. For each cheese frozen, there was a similar one that had been kept under ordinary storage conditions. Part of each cheese was processed within a week after it had been removed from the freezing temperature and the remainder was processed 6 weeks later after storage at 40° to 50°F. It was impossible to show any marked differences between the frozen and the unfrozen cheese during or after processing. With the former there appeared to be a slight fat separation with the younger samples as the heating started, but this was all re-incorporated before the cheese was drawn from the kettle. The results with

these cheeses confirmed those obtained previously with cheeses of similar age and reaction.

In connection with the work on the effects of age on the processability of cheese, some experiments were conducted on the distribution of the nitrogen between water and 5 per cent salt (NaCl) soluble. The results of these investigations are shown in table 1. The findings of Van Slyke and his co-workers (3) have been confirmed regarding the changes in the distribution of the nitrogen as the cheese ages. With the very young cheese, the salt soluble fraction was very much greater than the water soluble,

TABLE 1

*The effect of age and processing on the nitrogen distribution in cheese*

| AGE | UNPROCESSED CHEESE | | | | | | PROCESSED CHEESE | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | Total nitrogen | Water soluble | | Salt soluble | | $H_2O$ | Total nitrogen | Water soluble | | Salt soluble | |
| | | | Nitrogen | Per cent of total N | Nitrogen | Per cent of total N | | | Nitrogen | Per cent of total N | Nitrogen | Per cent of total N |
| *months* | *per cent* | *per cent* | *per cent* | | *per cent* | | *per cent* | *per cent* | *per cent* | | *per cent* | |
| 1 | 36.51 | 4.00 | 0.77 | 19.25 | 1.24 | 31.00 | 36.8 | 3.67 | 3.15 | 85.83 | | |
| 1 | 35.04 | 3.91 | 0.88 | 22.51 | 1.94 | 49.61 | 39.2 | 3.53 | 1.75 | 49.57 | 0.13 | 3.68 |
| 3 | 37.38 | 3.70 | 1.78 | 48.11 | 0.98 | 26.49 | 37.1 | 3.38 | 2.62 | 77.51 | 0.12 | 3.55 |
| 3 | 37.42 | 3.81 | 1.66 | 43.57 | 0.58 | 15.22 | 36.4 | 3.65 | 2.46 | 67.40 | 1.11 | 30.41 |
| 6 | 36.71 | 3.98 | 1.76 | 44.22 | 0.28 | 7.03 | 38.7 | 3.77 | 2.12 | 56.23 | 0.22 | 5.83 |
| 6 | 36.70 | 3.92 | 1.78 | 45.41 | 0.22 | 5.61 | 39.5 | 3.60 | 2.73 | 75.83 | | |
| 9 | 36.01 | 3.90 | 1.79 | 45.12 | 0.58 | 14.87 | 38.5 | 3.48 | 1.81 | 52.01 | 0.19 | 5.46 |
| 9 | 36.25 | 4.03 | 1.71 | 42.43 | 0.44 | 10.92 | 38.1 | 3.68 | 2.40 | 65.22 | 0.22 | 5.97 |
| 9 | 34.49 | 4.18 | 2.05 | 49.04 | 0.21 | 5.10 | 37.6 | 3.57 | 2.26 | 63.31 | 0.09 | 2.52 |

but as the age increased the water soluble portion increased, and the salt soluble decreased quite rapidly. Besides the work on the unprocessed cheese, determinations were made on the cheese as soon as possible after processing and the results are shown in the second part of the table. The results were very interesting in respect to the very significant increase in the amount of water soluble nitrogen that was found after processing. In every case there was a decided increase although the actual amount showed a rather wide variation. The average increase calculated as the per cent of total nitrogen is approximately 26 per cent.

SUMMARY

From the results obtained in this experimental work, there are a number of conclusions that may be drawn:

1. The use of the so-called emulsifiers is beneficial and the results obtained with sodium citrate indicate that it is superior to di-sodium phosphate. The use of more than 3 per cent of these salts should be avoided not only to remain within the legal limits but also for the effect on the finished product.

2. Temperature regulation is essential in order to insure the proper pasteurization of the cheese and also to prevent the color from changing to "salmon pink." If the cheese is to be held in the kettle for any length of time, it should be at the lower pasteurization temperature and water should be added to make up for the losses due to evaporation.

3. Close observance of the standards set for the moisture content of processed cheese are essential not only for economic reasons, but also to insure having cheese of as uniform body as possible.

4. Good results were obtained with cheese ranging in reaction from pH 5.8 to 6.2. This would indicate that this factor can be adequately controlled by exercising discrimination in blending.

5. Very young cheese showed excessive fat separation in processing, and a rubbery texture in the finished product. Very old cheese also processed unsatisfactorily producing a product with a weak body and grainy texture. Good results were obtained when the blending was such that the average age of the cheese was from 4 to 7 months.

6. A comparison of the reaction in terms of pH and the titratable acidity furnishes a means of getting some ideas as to the kind of cheese that was processed and the treatment that it received.

7. Cheese that can be cut in slices 0.02 inch thick and requires 800 to 1200 grams with a mechanical advantage of 5 to crush an inch cube to one-half of its original thickness may be considered as having a very desirable body.

8. The keeping quality of processed cheese is dependent upon

the heat treatment, the reaction and to some extent upon the temperature of the store-room.

9. Discoloration of the tin foil may be due to the use of phosphate as the emulsifier or to the use of alkali giving a reaction above pH. 6.3.

10. Frozen cheese can be processed satisfactorily.

11. Processing increases the water soluble form of nitrogen in the cheese.

In conclusion the authors wish to express their thanks to the Chas. Pfizer and Company, Inc., who have sponsored the fellowship under which this work has been done.

The analytical work on the nitrogen distribution was done with the assistance of Glenwood Mutten.

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# STUDIES ON OXIDATION-REDUCTION IN MILK

## THE METHYLENE BLUE REDUCTION TEST*

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In the first two papers of this series some theoretical aspects of oxidation-reduction phenomena in milk were discussed. The present paper deals with some of the more practical considerations of the methylene blue reduction test. The reader is referred to the previous articles (Thornton and Hastings, 1929) for a more complete discussion of the mechanism of reduction than space permits in this paper.

### METHODS

The water bath employed was electrically heated and thermostatically regulated. The temperature used was 37.5°C. ±1°, unless otherwise stated. The temperature in different parts of the bath did not vary more than one-half degree.

Except where noted 10 cc. portions of milk in test-tubes were used. The methylene blue tablets manufactured by the National Anilin and Chemical Company were used. Unless reported to the contrary one tablet was dissolved in 200 cc. of distilled water and this solution was sterilized by steaming. One cubic centimeter of this solution was added to 10 cc. of milk, giving an approximate concentration of 1 part of dye to 200,000 parts of milk (Standard Methods of Milk Analysis, 1928). Dye concentrations as reported in this paper are, therefore, only approximate.

The end-point of reduction was considered reached when the milk appeared white on closing the tube and inverting it once to mix the contents thereof. All reduction times are reported in hours and minutes. Thus, 13:07 means 13 hours and 7 minutes.

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The heading "Time" in the tables means reduction time. Care was taken to have all equipment sterile except in those cases calling for other technique.

In the plate count work Standard Methods of Milk Analysis (1923) were followed with the exception that 1 per cent of glucose was added to the medium and the counting was done without the aid of a reading glass. Three plates were poured from each dilution and not less than 2 dilutions were plated per sample. Incubation periods were for 2 days at 37.5°C.

## THE MECHANISM OF REDUCTION

Since there is no oxygen in the methylene blue molecule, the reduction of this dye involves a transference of hydrogen. The source of this hydrogen is still uncertain. It seems probably that many compounds act as hydrogen donators. These have been called metabolites. To these belong such substances as the sulfhydryl compounds, citrates, succinates, aldehydes, and others. Lactose must also receive consideration as a factor in reduction in milk.

Methylene blue may act as an acceptor for the hydrogen and undergo reduction. Molecular oxygen dissolved in the milk may also be reduced by the hydrogen, according to the theory of Wieland, first forming $H_2O_2$ which is then reduced to $H_2O$. Coincident with the oxidative and reductive changes in milk, potential changes are also observed. The potential values of fresh milk fall within a rather narrow range. As dissolved molecular oxygen disappears the potentials of the milk become more negative. At the more positive values the oxygen seems to have a greater affinity for the hydrogen than does methylene blue. When a certain potential is reached, the situation appears to be reversed and the hydrogen reduces the dye (Clark, Cohen, and Gibbs, 1925). The removal of oxygen by mechanical, chemical, or biological means causes a fall of potential in the milk. Barthel (1917), and Thornton and Hastings (1929) washed the molecular oxygen out of fresh raw, and sterile milk with inert gases and observed reduction of methylene blue. Barthel (1925) reports reduction of this dye in a sterile mixture of lactose, peptone, kaolin,

milk salts, and sodium citrate. Dubos (1929), and Thornton and Hastings (1929) studied the reduction of dyes in sterile bacteriological media. Coulter (1928) reported the oxidation-reduction potentials of sterile nutrient bouillon. Burri and Kürsteiner (1912) showed that reduction of methylene blue takes place in fresh milk in which bacterial action is inhibited by antiseptics. They thought that dissolved oxygen plays an important rôle in this test. Thornton and Hastings present evidence that milk itself consumes oxygen, a characteristic probably common to all organic complexes.

Barthel (1917) believed that the disappearance of methylene blue in milk takes place in two stages, viz.:

1. The removal of the dissolved oxygen by the growing bacteria
2. The reduction of the dye by constituents of the milk

There are today considerable data in support of this theory. It is probable that minute but inconsequential amounts of oxygen are fixed by constituents of the milk during the operation of the test. Some investigators believe that reduction of the dye takes place within, or at the surface of, the bacterial cell. We are of the opinion that at least the major portion of the dye is reduced independently of the cell. All of our work tends to confirm the theory of Barthel. Therefore, the reduction time of methylene blue in raw or pasteurized milk may be looked upon as a criterion of the oxygen-consuming power of the bacteria in the milk.

## A CONSIDERATION OF OXYGEN RELATIONSHIPS

The importance of the rôle of oxygen in the methylene blue reduction test has been recognized to the extent that some have mentioned the possibility of inaccuracy due to variations in the oxygen content of different milks. It has been advised that the milks be given a preliminary shaking to bring them into equilibrium with the atmosphere, and then be protected from the diffusion of atmospheric oxygen during the test by covering with vaseline or paraffin. We have shown in our previous papers that the rate of diffusion of atmospheric oxygen into whole milk is so slow as to have no measurable effect upon reduction times if the tests

are carried out in the usual culture tubes. The protective effect of the cream layer and of the aerobic flora, which is probably universally present, is sufficient to account for these results. In only one case did we find a difference between the reduction times of protected and unprotected tubes of skim milks.

Marshall (1902) reported the oxygen content of the gas from six samples of milk, obtained by the ordinary methods of milking, as being:

| Milk number..... | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Per cent oxygen.. | 13.79 | 12.66 | 14.17 | 11.89 | 10.96 | 15.57 |

The total amount of gas in these milks was fairly constant. Different methods of handling the milk subsequent to milking, such as aeration over glass, tin, copper, glass wool, etc., caused an appreciable increase in the oxygen content of the gas. There is likely to be, as these figures show, some variation in the oxygen content of different milks as received at the milk plant. The data which we are presenting show that inaccuracies in the test due to this variability are probably small.

Lojander (1925) claimed that shaking the milk 30 times results in a lengthening of reduction times. Other workers, as Hastings, Davenport, and Wright (1922) found that a preliminary shaking has practically no influence upon reduction times. We have not observed that preliminary shaking makes any appreciable difference in results.

An experiment in which the milk was shaken for 15 minutes is reported in table 4. In another experiment oxygen was bubbled through the milk for 5 minutes prior to the addition of the methylene blue. In neither experiment was the reduction time appreciably affected. These results showed that the original milk was in approximate equilibrium with the air. We have observed nothing leading us to believe that differences in the oxygen content of milks produced in the ordinary way will introduce serious inaccuracies into the test. It can be assumed, therefore, that the reduction times of methylene blue in milk, as observed in the standard operation of this test, are influenced almost entirely by the rate of oxygen uptake by the milk bacteria.

The rates of oxygen uptake by different species of bacteria are not well known (Callow, 1924; Burnett, 1927; and Soule, 1928). It is probable that the rates vary with different species, and with different conditions, sufficiently to introduce an important factor of inaccuracy into the test. This, we believe, is one of two important sources of inaccuracy, the second being the removal of bacteria from the body of the milk by the rising butterfat. A knowledge of the rates of oxygen uptake by bacteria would be of doubtful value, as far as this test is concerned, since the flora of the milks subjected to the test in practice is an unknown factor. This situation is relieved, somewhat, by the tendency of the lactic acid bacteria to predominate in most of the milks to which the test is applicable.

Barthel (1908) and Orla-Jensen (1912) reported the lactic bacteria to be slow reducers, while Fred (1912), Rahn (1920) and Hastings, Davenport, and Wright (1922) classed them among the strongly reducing organisms. The latter authors pointed out the reason for this divergency of opinion. Barthel and Orla-Jensen added methylene blue to cultures of the bacteria in which growth had already taken place. In the experiments of the other workers the dye was added before growth had been retarded. As we have already seen, the reduction of methylene blue in milk is the result of bacterial activity. Under the first condition the oxygen-consuming capacity of dormant bacteria was in question; under the second the capacity of growing organisms was in question. The rate of oxygen uptake by reproducing cells is several fold that of dormant cells.

The bacteriologist frequently uses plant and animal tissues to produce anaerobic conditions. The oxygen-consuming and the reducing properties of living plant and animal tissue are recognized. It is logical, therefore, to suppose that leucocytes have this power and that they are a factor in the reduction of methylene blue in raw milk. Cases have been reported in which the reduction times of certain samples of milk have been disproportionate to the bacterial content of the milk as revealed by the plate or by direct microscopic counts. Skar (1913) studied this question and established the reducing power of cells from the

lymph glands and of leucocytes from centrifuged milk. A mathematical relationship between leucocytes and reduction times has not been established as has the relationship between bacteria and reduction times. The effect of age and temperature upon the reducing activities of these body cells is not known. It is improbable that the leucocytes play a major rôle in the reduction test except, perhaps, in long-time reducing or abnormal milks.

## THE EFFECT OF VARIATION OF pH

Mueller (1906) showed that the neutralization of considerable produced-acid in milk does not change its reduction time. This

TABLE 1

*Plate counts in millions taken at the moment of reduction*

| ½ HOUR REDUCTION TIME | ½–1 HOUR REDUCTION TIME | 1–2 HOURS REDUCTION TIME | 2–3 HOURS REDUCTION TIME | 3–4 HOURS REDUCTION TIME | 4–5 HOURS REDUCTION TIME | 5–6 HOURS REDUCTION TIME |
|---|---|---|---|---|---|---|
| 38 | 35 | 16 | 3.5 | 44 | 25 | 35 |
| 19 | 35 | 12 | 11 | 13 | 10 | 45 |
| 80 | 31 | 8 | 23 | 20 | 32 | 4.5 |
| 32 | | 17 | 16 | 5 | 19 | 5.5 |
| | | 26 | 23 | 20 | | |
| | | 12 | 22 | 29 | | |
| | | 9.5 | | 20 | | |

has been confirmed by Orla-Jensen. In their series of papers on oxidation-reduction, Clark and his coworkers showed that at 30°C. the potential becomes more negative by 0.06 volt for each increase in pH equivalent to unity. The figures presented in our first papers of this series show that the change of pH during the operation of the test is slight. It is seen that any variations in pH met with in milks to which this test is applicable are not sufficient to have any measurable visual effect upon reduction times. This is precisely what one would expect, for it is well known that the rate of growth of the lactic bacteria is not checked until the milk has developed sufficient acid to cause curdling at ordinary temperatures. Hastings and Evans (1913) reported that the number of bacteria must reach approximately 100 million be-

fore any appreciable change is noted in the reaction of the milk as measured by titration.

The plate counts of 39 samples of milk, made at the moment of decolorization, are given in table 1. One sample, giving a plate count of 80 million, was on the verge of souring and reduced methylene blue in less than 5 minutes. With this exception the counts varied from 3.5 million to 45 million with an average of 22 million. Because of the wide variations in the plate count these differences are probably more apparent than real. Nevertheless, wide variations are likely to occur. That the differences between the numbers present in the long and the short time reducing milks are not constant or striking may be significant. This may indicate that resting cells have little effect upon reduction times in the usual operation of the test. These results show conclusively that the bacterial content at the moment of reduction, while high, is not sufficiently so to cause any great change in the reaction of the milk.

### THE EFFECT OF TEMPERATURE

The relationship of temperature has been studied extensively by Rahn (1920) and by Hastings, Davenport, and Wright (1922). The ordinary water-bath is easily regulated, even when an alcohol lamp is used as the source of heat, so that temperature fluctuations need not be greater than 1 or 2 degrees. The work to which reference is made above points to the conclusion that inaccuracies in this test due to such small fluctuations in temperature would be comparatively small.

### THE SWEEPING EFFECT OF THE RISING BUTTERFAT

When working with this test, one soon observes the irregular and uneven disappearance of the blue color from certain samples of milk. Sometimes the major portion of the milk will turn white leaving a blue band at the top, bottom, or in the middle. In a few cases white streaks or patches appear. Difficulty is experienced in obtaining a definite end-point. Vitoux (1920) recommends disregarding the upper quarter in reading the test. Another method involves closing the tube with the finger, inverting

once, thus mixing any unreduced dye persisting in any part of the tube with the entire mass of milk. If the milk appears white the end-point is considered to have been reached. The slight amount of oxygen incorporated in the milk by the inversion of the tube has no significant effect on the reduction time, as more violent agitation may have. It is frequently difficult to tell the exact end-point even in cases in which the disappearance of the dye is uniform. Much depends upon the sensitivity of the eyes of the observer to blue light.

Many workers have reported the reduction times to 3 minutes. Except in very rapidly reducing milks we have found it difficult to read the end-point, in many cases, to within 5 and even 10 and 15 minutes. In very slowly reducing milks this doubtful period is extended greatly. In one sample the end-point was taken as 25 hours, but it was simply guess work after 23 hours. Experience soon enables one to judge the end-point by the above method with a minimum of error. No pretense is made in this report that reduction times are correct to within 5 minutes except in the case of rapidly reducing milks. In the majority of cases times are reported to the nearest 15 minutes.

In attempting a determination of the accuracy of this test wide variations in the reduction times of duplicate tubes were noted. It was observed that these wide discrepancies appeared in the exceptionally good milks. Smaller and less serious variations were noted in some of the more mediocre milks. We have endeavored to find the cause for this and the results here reported are representative of a large number of such experiments. In one experiment 36 duplicate tubes were prepared in the standard manner. Of another sample of milk only 5 tubes were used. The results of the experiments are given in table 2.

These variations, we believe, are due to the uneven sweeping of the bacteria out of the milk by the rising butterfat. Skar (1913), observing this same variation, attributed it to the bacteria and the leucocytes being carried out of the milk by the butterfat and to a settling of some of the cells to the bottom of the tube. Not a great deal of stress has been laid on the importance of this source of inaccuracy in the reduction test.

Anderson (1909) found that gravity cream contained on the average approximately 15 times as many bacteria as the skim milk. Schmidt (1925) reported the lactic acid bacteria 85 times more numerous in the top than in the lower layers of milk. Leete (1926) gave the following figures as the average of 25 samples:

| | *Number of bacteria per cubic centimeter* |
|---|---|
| Whole milk | 135,880 |
| Gravity cream | 283,680 |
| Skim milk | 33,556 |

TABLE 2

*Reduction time variations in duplicate tubes of two milks*

| SAMPLE 1 | | SAMPLE 2 | |
|---|---|---|---|
| Number of tubes | Time | Number of tubes | Time |
| 3 | 9:30 | 1 | 13:15 |
| 2 | 9:45 | 1 | 13:30 |
| 2 | 10:00 | 1 | 16:00 |
| 3 | 10:15 | 1 | 21:45 |
| 12 | 10:30 | 1 | 25:00 |
| 1 | 10:45 | | |
| 2 | 11:00 | 5 | Ave. 17:54 |
| 8 | 11:15 | | |
| 1 | 11:30 | Variation 11:45 | |
| 1 | 12:00 | | |
| 1 | 12:30 | | |
| 36 | Ave. 10:48 | | |
| Variation 3:00 | | | |

Breed (1926) in working on the effect of gravity creaming on the body cell content of the cream and skim milk reported a tremendous sweeping of cells into the cream layer. In commenting on his results Breed writes,

These examinations show that when cream is allowed to rise by gravity the greater portion of the cells rise with the cream. The reason for this is simple. Many of the cells contain fat drops and therefore naturally tend to rise with the cream. Others of the cells adhere to fat drops and these are buoyed up with the fat drops as they rise. These

observations show that gravity cream may be invariably expected to contain large numbers of cells as the cells concentrate in the cream when it is formed in this way. . . . . Skim milks invariably contain few cells.

A simple experiment to prove this is to allow some tubes of good milk to sour. If half of these tubes are shaken frequently, while half are left undisturbed, the former will coagulate first.

If the above mentioned variations in the reduction times of duplicate samples of better class milks are due to the rising of the bacteria with the butterfat, then these variations should be lessened or even entirely wiped out if creaming is prevented. We have used different methods to accomplish this. The following

TABLE 3

*Effect of shaking on reduction time*

| UNSHAKEN | | SHAKEN | |
|---|---|---|---|
| Number of tubes | Time | Number of tubes | Time |
| 1 | 10:15 | 1 | 6:50 |
| 1 | 10:50 | 1 | 6:50 |
| 1 | 11:05 | 1 | 7:05 |
| 1 | 11:10 | 1 | 7:05 |
| 1 | 12:05 | 1 | 7:05 |

are representative of a large number of experiments which have been made. Five duplicate tubes were incubated at rest and 5 were shaken every 15 minutes during the entire course of the test. The results are presented in table 3.

If the difference between the shaken and unshaken tubes is due to keeping the bacteria mixed with the milk, then it should make little difference whether the shaking is violent or gentle, frequent or infrequent (within certain limits). The following results are indicative of this. In the case of a milk showing a reduction time of 8 hours, 5 tubes shaken gently (2 or 3 times) every half hour reduced in 6:30, 5 tubes shaken violently (25 times) each half hour reduced in the same time. Similar results were obtained in a second trial with another milk.

It frequently happens with poor milks and occasionally with middle class milks that shaking increases the reduction. With these milks quarter-hour and half-hour shakings give different results. This is due to the incorporation of oxygen just at the time of decolorization, at which time the oxygen content of the milk is very materially lowered.

TABLE 4

*The effect of preliminary shaking with glass beads on reduction time*

| NOT SHAKEN WITH GLASS BEADS | | SHAKEN WITH GLASS BEADS | |
|---|---|---|---|
| Unshaken in bath | Shaken in bath | Unshaken in bath | Shaken in bath |
| *time* | *time* | *time* | *time* |
| 10:15 | 6:50 | 9:05 | 6:35 |
| 10:50 | 6:50 | 9:25 | 6:35 |
| 11:05 | 7:05 | 10:15 | 6:35 |
| 11:10 | 7:05 | 10:15 | 6:35 |
| 12:05 | 7:05 | 10:20 | 6:35 |

TABLE 5

*Reduction times when the milk was coagulated by rennet*

| SAMPLE NUMBER | UNSHAKEN CONTROLS | SHAKEN CONTROLS | PLUS RENNET |
|---|---|---|---|
| 1 | 0:15 | 0:15 | 0:15 |
| 2 | 4:00–4:10 | 3:30 | 3:00 |
| 3 | 1:45–2:05 | 1:15–1:20 | 0:50 |
| 4 | 5:15 | 4:15 | 4:00 |
| 5 | 3:45 | 2:30 | 2:00 |
| 6 | 1:30 | 1:30 | 1:05 |
| 7 | 4:45 | 3:45 | 3:00 |

In order to find if the shaking was breaking up the clumps of bacteria and distributing them more evenly throughout the milk, thus effecting a more uniform action, or if the more uniform results were due merely to keeping the organisms in the milk, the following experiment was performed. A sample of milk was divided into two portions. One portion served as a control and was left untreated. The other portion was shaken with sterile glass beads for 15 minutes. A microscopic examination showed that incipient churning had taken place. Five tubes of each

portion were submitted to the test in the usual way. Five tubes of each lot were shaken half-hourly while in the water bath. The results are given in table 4. In view of the fact that incipient churning had taken place, it would not be expected that the sweeping action in this portion would be as great as in the normal milk. This experiment shows that the effect of shaking is due in large part to keeping the bacteria in the milk.

In another series of experiments 1 cc. of rennet was added to 200 cc. of the standard methylene blue solution and 1 cc. of this was added as usual to 10 cc. of milk. As controls, tubes were also run with the standard methylene blue solution without the rennet. The rennet milk coagulated within a few minutes. It was impossible to make accurate observations on the long time reducing milks owing to the shrinking of the curd. In the work

TABLE 6

*Reduction times when the milk was coagulated by agar*

| TREATMENT | NUMBER OF TUBES | AVERAGE TIME | VARIATION |
|---|---|---|---|
| Unshaken | 18 | 12:42 | 3:15 |
| Shaken | 18 | 10:40 | 0:30 |
| Plus agar | 9 | 9:00 | 0 |

reported in table 5, 5 tubes were used in each case. The concentrated rennet solution itself reduced methylene blue. Nevertheless it seems unlikely that, in the concentration used in these milks, any appreciable reducing effect would be noticeable. The divergency between the shaken tubes and the rennet tubes was possibly due to the incorporation of oxygen in the former at the moment of reduction.

In the next series of experiments the milk was coagulated by the addition of 1 cc. of sterile 3 per cent agar to each 10 cc. of milk. Results are given in table 6. The tubes tabulated under "Shaken" were incubated undisturbed for the first 9 hours of the test. This may account for the difference between the reduction times of these tubes and of the agar tubes.

To demonstrate that the lessened reduction times were not

due to any reducing action of the agar itself 5 tubes each of 10 cc. portions of the same milk containing varying amounts of the agar were tested with the results as shown in table 7. In adding agar to the milk one is adding extra oxygen for the same number of bacteria to consume. A foreign colloid is also being added. In the light of Virtanen's experiments (1924) and of our own, neither of these additions should have any measurable effect upon the reduction time when only 1 cc. of 3 per cent agar is added to 10 cc. of milk. The following experiment proves that this con-

TABLE 7

*The effect on reduction time of adding varying amounts of agar to the milk*

| AMOUNT OF AGAR SOLUTION | TIME |
|---|---|
| *cc* | |
| 1 | 9:00 |
| 2 | 9:15 |
| 3 | 9:20 |
| 5 | 9:45 |
| 10 | 10:15 |

TABLE 8

*The effect on reduction time of adding agar solution and water to milk*

| | UNSHAKEN | SHAKEN |
|---|---|---|
| | *time* | *time* |
| Whole milk | 0:45 | 0:45 |
| Agar and milk | 1:10 | |
| Water and milk | 1:00 | 1:00 |

tention is true. Milk was divided into 3 portions. To one part an equal quantity of agar was added. To another was added an equal quantity of sterile water, while the third was left untreated. The reduction times of a number of duplicate tubes of each of these are given in table 8.

In the next experiment milk was placed in small bore tubes so bent that the central portion of the tubes rested horizontally upon the floor of the bath while at each end a vertical portion extended to the surface of the water. In the horizontal parts of the tubes the butterfat could rise but a short distance, while in the vertical

parts the usual condition obtained. The milk in all the horizontal portions decolorized in 11 hours and 15 minutes without variation while decolorization in the vertical ends extended over a period from 12 hours and 30 minutes to 16 hours.

An experiment was made in which the butterfat was kept from rising in the milk by homogenization. A fresh milk was run through a clean homogenizer without the application of pressure. The milk was put through again under a pressure of 2500 pounds. It was run through the third time under a pressure of 3500 to 4500 pounds. The usual technique of the reduction test was then used. The results of 15 duplicate tubes in each case are presented in table 9.

TABLE 9

*The effect of homogenization on reduction time*

| | UNSHAKEN | | SHAKEN | |
|---|---|---|---|---|
| | Average time | Variation | Average time | Variation |
| Unhomogenized | 10:24 | 1:00 | 6:30 | 0 |
| First homogenization | 6:45 | 0 | 6:49 | 0:10 |
| Second homogenization | 6:47 | 0:05 | 6:47 | 0:05 |

It is to be noted that the two means, homogenization and mixing at intervals during the test, of keeping the fat and bacteria from concentrating in the upper layers of the milk have produced the same results.

It has been found that shaking, or any method which keeps the bacteria in the milk, not only frequently shortens the reduction times and wipes out the variations often noticed, especially in good milks, but in all cases causes uniform disappearance of the blue color throughout the whole tube.

These experiments show the sweeping effect of the rising butterfat and provide an explanation for the uneven disappearance of the dye from the individual tubes and for the variations in duplicate tubes. The sweeping effect is less at the bottom of the tube and increases upward. Skar suggests that there may also be a settling of the bacteria and leucocytes to the bottom of the tube.

The effect of the concentration of the bacteria in the upper layers is frequently seen since reduction often occurs here before, or simultaneously with, reduction at the bottom. The cream layer almost invariably decolorizes first. This leaves a portion in the middle of the tube containing, in comparison, few bacteria, thus leaving a blue band in this region. Diffusion of oxygen is necessarily very slow and one portion of the tube has little effect upon distant parts. Since the factors involved are physical and not chemical, duplicate samples will vary in the number of bacteria left in the milk. This accounts for the variations in the reduction times of duplicate tubes and for the white and blue regions and patches so frequently observed. Large variations have been confined almost entirely to good milks of low bacterial content. This is what one would expect since a larger percentage of bacteria is likely to be swept out of such a milk than out of milk of high bacterial content.

Shaking the milk during the period of incubation introduces two sources of error. There is the possibility of the incorporation of oxygen just at the moment of reduction. We found that shaking every 15 minutes or just at the end of the test prolonged the time required for reduction from 15 minutes to 1 hour. In working as much care as possible was taken to avoid this error. In long-time reducing milks this added reduction time is small in comparison to the total reduction period and is much more than counter-balanced by the effect of keeping the bacteria down in the milk. There is also the possibility of the aeration affecting the rate of growth of the bacteria. Little is known regarding this effect. Rogers and Whittier (1927) reported that the level of growth of Str. lactis is higher on aeration. This factor cannot be operative in the cases here reported except, perhaps, during the latter part of the reducing period and would thus be insufficient to exert any appreciable effect. The data here cited are indicative of this.

In order to learn the magnitude of the effect due to creaming on reduction time in milks of varying bacterial content 95 samples of milk were examined. Tubes of each were allowed to remain undisturbed. Duplicate tubes were inverted at 30-minute in-

tervals. Variations of less than 15 minutes were not considered. The maximum variation in the case of unshaken tubes was 2:30 on a milk of the 10–12-hour group, in the "shaken" group 15 minutes in one of the 8–9-hour group. The effect of keeping the bacteria uniformly distributed in the milk during the test is most evident from the data of table 10, both as regards constancy of reduction time and length thereof. The relation between opportunity for creaming, due to longer reduction periods, and increased reduction time is evident from the last column of the

TABLE 10

*The effect of shaking samples of milk during incubation on length and constancy of reduction time*

| TIME OF REDUCTION | NUMBER OF SAMPLES | NUMBER OF SAMPLES SHOWING VARIATIONS IN DUPLICATE TRIALS | | AVERAGE DECREASE IN REDUCTION TIME DUE TO SHAKING |
|---|---|---|---|---|
| | | Unshaken | Shaken | |
| *hours* | | | | *hours* |
| 10–12 | 5 | 5 | 0 | −3.00 |
| 9–10 | 5 | 5 | 0 | −2.5 |
| 8–9 | 8 | 4 | 1 | −2.0 |
| 7–8 | 10 | 3 | 0 | −1.1 |
| 6–7 | 8 | 1 | 0 | −1.5 |
| 5–6 | 9 | 1 | 0 | −1.5 |
| 4–5 | 10 | 1 | 0 | −1.4 |
| 3–4 | 11 | 1 | 0 | −0.7 |
| 2–3 | 7 | 0 | 0 | −0.25 |
| 1–2 | 11 | 2 | 0 | −0.20 |
| −1 | 11 | 0 | 0 | 0.00 |

table. Consideration was given to the amount of fat in the milks tested. No observations were made which indicate any marked parallelism between the increased reduction time and high butterfat content of the milk.

It does not seem wise to suggest any modification of the test as described in the last edition of "Standard Methods for Milk Analyses," 1928. It is evident that mixing at intervals during the incubation period will lead to more accurate results with good milks. With the poorer milks the effect on accuracy of mixing would be slight. It should be remembered that the test is being

used to divide milks of widely varying bacterial content into a few groups, rather than to differentiate between milks of approximately equal bacterial content. The test will be most valuable as a control agent when kept as simple as possible. The technique of mixing should probably be introduced in any attempt to correlate the reduction time of good milks with the results supplied by any other method of determining bacterial content.

### THE USE OF A MEASURING DIPPER

"Standard Methods of Milk Analysis" calls for the use of steamed or sterilized 10 cc. pipettes for measuring the samples of milk to be tested. In the routine application of the test this practice has been found to be cumbersome and lessens its simplicity. In some milk distributing plants and cheese factories a 10 cc. dipper has been adopted in the place of the pipettes. At least one manufacturer has such a dipper upon the market.

To determine if such a method is permissible experiments were performed upon a number of samples of milk of all classes. No difference in reduction times could be observed between those samples pipetted and those measured with the dipper. It was found that under careful laboratory conditions such a dipper measured water with less variation than the various 10 cc. pipettes picked up at random in the laboratory. This accuracy, however, is unlikely in the weigh room of a milk plant or cheese factory. Scrutiny of our results on the effect of dye concentration on reduction times (Thornton and Hastings, 1929) will disclose the fact that extreme accuracy in measuring the sample is not necessary. Rather wide variations will give identical results. The reason for this has been explained. Therefore a 10 cc. dipper is sufficiently accurate for use in this test.

### STERILITY OF DIPPER AND DYE SOLUTION

If non-sterile apparatus will give the same results as sterile equipment, then the use of the former will add materially to the attractiveness of this test in the mind of the practical man. A study of this question was made and the results of two experi-

ments are given. Two samples of incoming milk were each collected in a quart bottle. A sample was taken out of each for a control. After taking each measureful of these milks the dipper was rinsed twice in a 12-quart pail of cold water, then dipped in a sample of milk which was just sour to the taste and which had a reduction time of less than 5 minutes. The dipper was then rinsed twice in the pail of cold water before being used to measure the next sample. Sixteen samples of each milk were taken which would be the equivalent of collecting 64 samples in the receiving room every other sample being sour. No influence on the reduction time was noted with either milk, all samples of milk 1 reducing in 1:15, of milk 2 in 4:45.

This experiment was repeated using two other milks and 40 samples of each were measured out. The technique of immersing the dipper in a sour milk and rinsing between each sample was identical with the last experiment. This was the equivalent of collecting 160 samples in the weigh-room. No evidence was obtained which indicated that the bacterial content of the samples taken at the end of the sampling was greater than at the beginning.

The conditions under which these tests were made were so much more extreme than one would meet in practice that we are justified in concluding that, in the routine application of the test, it is sufficient to rinse the dipper in the weigh-tank or can of milk about to be sampled if readings are not made after the 5½-hour period. Up to 0.25 gram of milk will cling to a smooth dipper of this size after the ordinary emptying. If this were a milk containing 4 million bacteria per cubic centimeter and were mixed with a can of milk, but 25 bacteria per cubic centimeter would be added to this next sample. This makes the results of our experiments seem reasonable.

A study was made of the effect of sterilizing the methylene blue solution as follows: Sterile dye was added to 30 tubes of milk and incubated. To 30 tubes of the same milk a non-sterile dye was added. The water used for dissolving the dye in this experiment was one not considered fit for drinking purposes. The average reduction time for the sterile dye was 8:58 with a variation between extremes of 45 minutes. The average reduction time for the non-sterile dye was 9:21 with a variation of 45 minutes.

In another experiment 10 tubes of milk contained sterile methylene blue solution and 10 tubes of the same milk contained dye solution which had stood exposed to the laboratory air for one month. The average reduction time for the sterile dye was 5:09 with a variation of 10 minutes. The average for the non-sterile dye was 5:54 with a variation of 20 minutes.

In the routine application of the test the use of any water fit for drinking is warranted as a solvent of the dye if the reduction times are not to be read after the 5½-hour period.

### THE PLATE COUNT AND THE REDUCTION TIME

A vast amount of work has been done in attempting to find the correlation between the methylene blue reduction test and the bacterial content of milk as shown by the plate count. Indeed, most of the workers in recent years have done a certain amount of this sort of investigation. The purpose of such investigations has been twofold—to give us a comparative classification of milks and a knowledge of the accuracy of this test.

Those experimenters who regarded the plate method as an approximate one only have been inclined to judge the methylene blue test sufficiently accurate to be of much practical value. Those who have accepted the plate count as something approaching infallibility have doubted the value of the reduction test. The more or less wide spread feeling that these two methods should give almost identical results, failing which one test or the other should be condemned, is unfortunate. The two methods do not measure the same thing. The plate method is the measure of the number of clumps of bacteria in a minute portion of milk which will grow under the conditions imposed in making such an analysis. The methylene blue reduction test is a measure of bacterial activity in milk itself under the conditions imposed in this test. One could not reasonably expect the two methods to agree exactly. Because in many cases they have not agreed, the reduction test has been called a "rough" test. Some seem to fear that an effort is being made to supplant the plate count. Surely these can have small appreciation of the difficulties surrounding milk control. No one method of bacteriological analy-

sis should *supplant* another. One method should *supplement* the other.

At one time or another the reduction test has been expected to be a measure of the numbers, kinds, pathogenicity, and virulence of the bacteria in the milk as well as an indicator for dirt, acidity, and preservatives. It would tax all the methods known to the milk technologist to fulfill such expectations. The confusion in which one finds this subject has not been lessened by the failure of many experimenters to use uniform or standard techniques. Kufferath (1919) used a dye concentration of 1:3000–4000, Fred (1912) 1:10,000, while most of the recent work has been done with a concentration of 1:200,000. The same holds true for plating methods. Fred used Heyden-Nährstoff agar with an incubation period of 12 days. Barthel (1917) used gelatine and incubated the plates at room temperature for 6 days. Many workers neglect to report their techniques.

Jensen, Barthel, Schroeter, and Fred have all proposed classifications based upon the plate count. That accepted in this country is the classification of Barthel and Jensen (1912) and is given in "Standard Methods of Milk Analysis" (1928).

Rahn (1920) thinks this classification is not justified by the results from which it was evolved. In an analysis of Barthel's figures he finds discrepancies in 33 per cent of the cases. Twenty-three per cent of these inaccuracies he attributes to errors in the plate counts and 10 per cent to errors in the methylene blue test. He used the microscopic count as his measure of accuracy. Lind (1920) reported having made comparative tests on 1600 samples with results favorable to the reduction test. Bolling (1924) reported that, of "hundreds" of samples tested, only 1 per cent were placed in different classes by the two methods. The classes he used were those of Barthel just cited. His results seem remarkable. Hastings (1919) concluded

> It seems evident from the data submitted that the reduction test determines the number of bacteria as accurately as can be done by any other method. . . . . No one who has had experience with plate culture work would consider it possible to place in the correct order samples of milk, such as are presented, even when working with great care, to say nothing of using the ordinary routine methods.

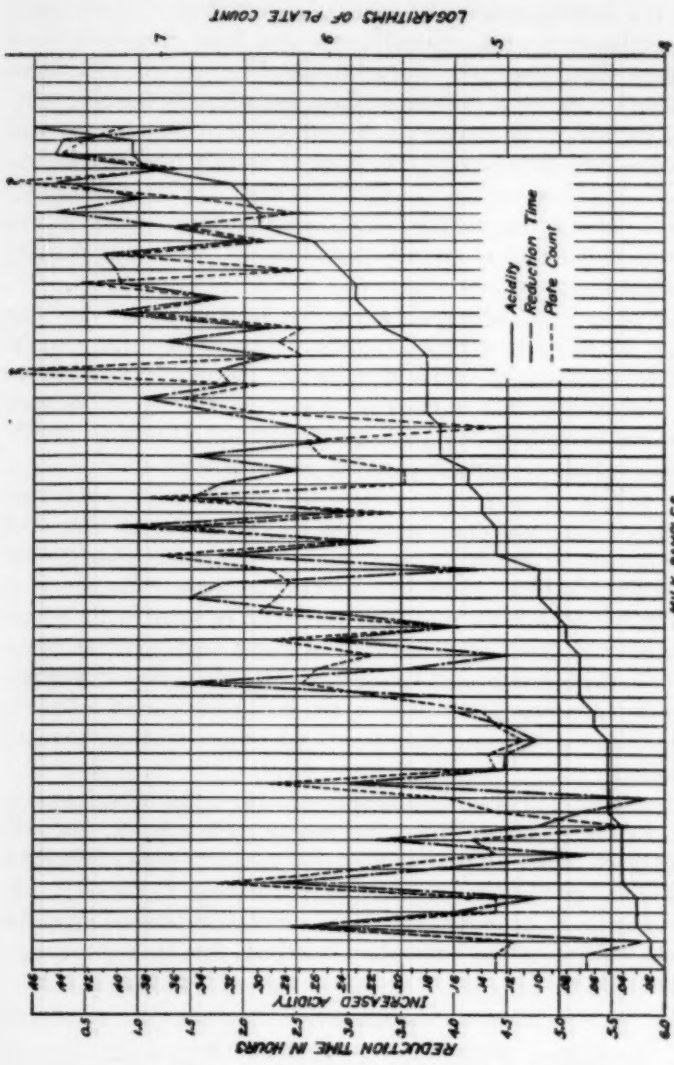


FIG. 1. SHOWING THE RELATIONSHIP OF REDUCTION TIME AND PLATE COUNT TO INCREASED ACIDITY OF 60 SAMPLES OF MARKET MILK

In the development and testing of any method of determining the number of bacteria in milk, attempts have commonly been made to show that the results obtained correlated with those supplied by the plate culture method and with the keeping quality of the milk. The latter is a difficult thing to define or to measure, involving as it does both qualitative and quantitative aspects. The increase in acidity has been the most common measure of keeping quality. This may or may not be a true measure in the case of an individual sample. It is quite certain to be true in general.

The plate count measures a varying fraction of the bacteria present in different samples of milk. The method has certain inherent inaccuracies, the nature and magnitude of which have been the subject of a number of recent papers. A preliminary report on the subject was made by Wright and Thornton (1927). The magnitude of variations noted and a general consideration of the subject of correlation of keeping quality, plate count and reduction time indicated the uselessness of making an extended study of milk to determine the relation of the results obtained by one method to those yielded by another method. The observations which have been made are presented in figure 1, in which the acidity is given in percentage of lactic acid, and the plate counts in the logarithms of the counts. It is to be noted that the trend of the curves is the same in all, with wide variations in reduction time and plate counts from each other and from increase in acidity.

It is probably impossible to divorce the two tests, reduction and plate, in the minds of those engaged in milk control. It will probably be found ultimately desirable to allow each method to stand alone, giving such information as each can to the user. It seems unwise to attempt any closer correlation than is given in the last edition of the "Standard Methods for Milk Analysis" (1928).

## SUMMARY

The mechanism of reduction is discussed. The work reported in this paper tends to confirm the theory of Barthel that the disappearance of methylene blue in milk takes place in two stages, viz.:

1. The removal of the dissolved oxygen by bacteria.
2. The reduction of the dye by constituents of the milk.

The time taken for the first stage may be long. The time taken for the second stage is usually short.

The oxygen relationship is of great importance. We were unable to find that a preliminary shaking of the milk materially affects reduction times. Evidence is presented that milks produced normally are in approximate oxygen equilibrium with the atmosphere.

In milks to which this test is applicable, the variations in pH are insufficient to cause a measurable difference in reduction times.

The importance of the rôle played by the leucocytes is not known.

The plate counts of 38 samples of milk taken at the moment of reduction varied from 3.5 million to 45 million with an average of 22 million.

It is probable that the two most important sources of inaccuracy are:-

1. The different rates of oxygen uptake of different species of bacteria.
2. The sweeping of bacteria out of the body of the milk by the rising butterfat during the operation of the test.

It is probable that the inaccuracies in this test follow the growth curve of the bacteria. Therefore, we do not consider the test reasonably accurate after the 5½-hour period as laid down in "Standard Methods of Milk Analysis."

Since the methylene blue is, indirectly, an indicator for oxygen, different concentrations of the dye, within limits defined in a previous paper, give identical reduction times. A 10-cc. dipper is, therefore, sufficiently accurate for use in measuring samples for this test.

It is sufficient that this dipper be thoroughly rinsed in the incoming milk or in water between each sample, if times are not to be read after the 5½-hour period. The use of any water fit for drinking purposes is warranted as the solvent for the dye.

The methylene blue reduction test is as accurate a measure of

the keeping quality of milk as any method yet available. It will divide milks into three or four classes with reasonable accuracy, as will any of a number of tests for milk quality. It is inexpensive and as nearly fool-proof as any method for this purpose available to the dairy bacteriologist. Methylene blue reduction times should not be reported in terms of the plate count.

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# STUDIES ON THE NUTRITIVE VALUE OF MILK

## IV. THE SUPPLEMENTARY VALUE OF YEAST IN NUTRITIONAL ANEMIA OF ALBINO RATS*

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In attempting to study the nutritive value of milk obtained from various sources by the exclusive milk method of feeding, nutritional disaster, including anemia, was encountered in rats at such an early age that no reliable data as to growth could be obtained. A systematic search for possible clues was undertaken, the results of which were previously reported (1). Various substances known to carry the essentials of a complete diet were added singly to the milk diet. As a source of the vitamin B-complex, yeast from our stock supply was used. The rats receiving this thrived; growth was excellent and hemoglobin remained at a high level. This could not be attributed to vitamin B (complex) as it had been previously shown that 16 cc. of milk was adequate when fed in addition to a basal ration free from this factor and the rats here were fed milk ad libitum. Furthermore, rice polishings, also supplying the B-complex, were not beneficial. Moreover, the ash of the same yeast was equally effective, suggesting that the factors involved were inorganic in nature.

Since iron had always been associated with hemoglobin, an analysis of the yeast for iron was made. The publication of the work of Hart, Steenbock, Waddell and Elvehjem (2) at this time suggested copper as a factor in hemoglobin formation. Analysis of the yeast for this element was also made. The sample was found to contain 0.0923 per cent iron and 0.0032 per cent copper.

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† Most of the chemical analyses were made by Miss Dorothy Woodland of Wooster College.

This meant that the daily intake from 0.4 gram of yeast was 0.3692 mgm. of iron and 0.0128 mgm. of copper.

Figure 1 shows the effect of the addition of 0.4 gram of stock dried yeast to an exclusive milk diet, both as a prophylactic and a

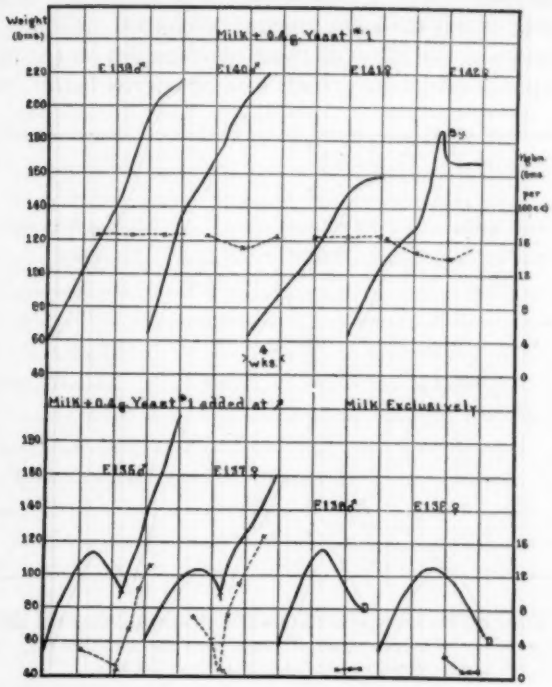


FIG. 1. The remarkable prophylactic and curative properties of yeast 1 are shown in this chart. This sample was rich in iron (0.0923 per cent) and contained a fair amount of copper.

In this and succeeding charts the solid lines signify weight and the broken lines hemoglobin.

curative substance. The curves of two representative rats not receiving the supplement are given for comparison.

The stock supply of yeast having become exhausted, a new supply was obtained from the same source. When 0.4 gram of

this supply was used to supplement milk the results were quite different from those obtained with the first supply. Growth was excellent, but the hemoglobin level became lower and lower until the rats were anemic. Analyses showed 0.0178 per cent iron and 0.0035 per cent copper, considerably less iron than in the first sample, and about the same amount of copper. Figure 2 shows the difference in the effect of these two samples on the hemoglobin of representative rats, both sets of curves being compared

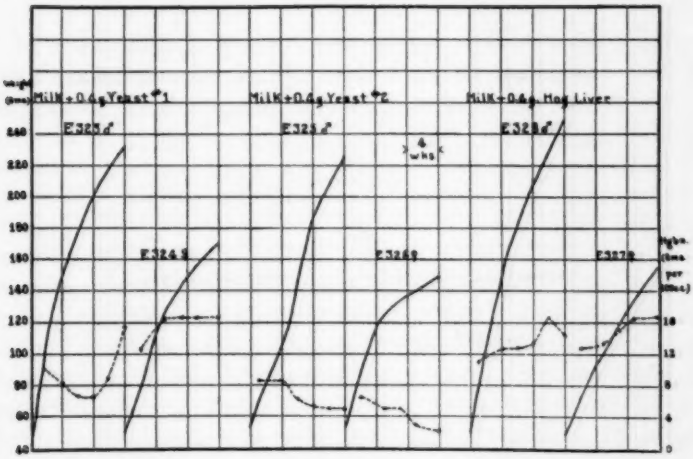


FIG. 2. These curves are representative of a number showing the difference in antianemic potency of two samples of yeast from the same source. The chief difference in chemical composition existed in the iron content.

with the curves for rats receiving an equal weight of dried hog liver.

A third sample of yeast from the same source having given still different results lying between those of the first two samples, it was decided that before any conclusion as to the value of yeast in general as an antianemic substance could be made samples from various sources must be assayed. Consequently a number of samples were obtained from the leading manufacturers of yeast and their antianemic potency determined.

## EXPERIMENTAL

The technique used in making the hemoglobin determinations, and the routine procedure of feeding, weighing, and caring for the rats have already been described (3). Ten samples of yeast were obtained from 6 manufacturers. Most of the samples were received in the dry form. One sample was delivered to the laboratory in fresh cakes, and another arrived in the form of a

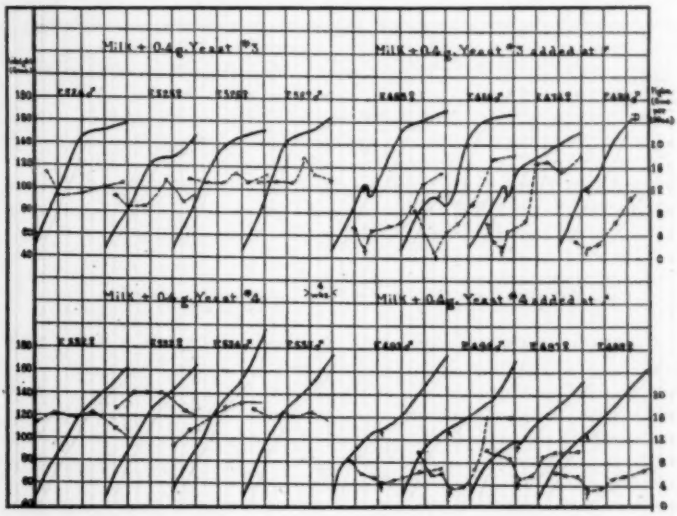

FIG. 3. Yeast 3 came from the same source as yeasts 1 and 2. It contained more iron than yeast 2 and more copper than either 1 or 2.

paste. These were dried and pulverized. Each sample was analyzed for dry matter, total nitrogen, ash, copper, and iron. The Official Methods were used for dry matter, total nitrogen, and ash. The Biazzo method (4) was used for copper, and the method of Fowweather (5) for iron.

Each sample was tested as a prophylactic and as a curative. In the prophylactic assay, 0.4 gram of dried sample was added daily to the milk from the beginning of experimental feeding.

The experiment continued for 16 weeks. In the curative assay the hemoglobin of the rats was allowed to drop to from 2 to 4 grams per 100 cc. of blood, at which time 0.4 gram of yeast was added daily. The fortified ration was continued until 16 weeks had elapsed from the beginning of the experiment. Hemoglobin determinations were made as a rule every 2 weeks. In some cases where a critical condition seemed imminent hemoglobin determinations were made oftener.

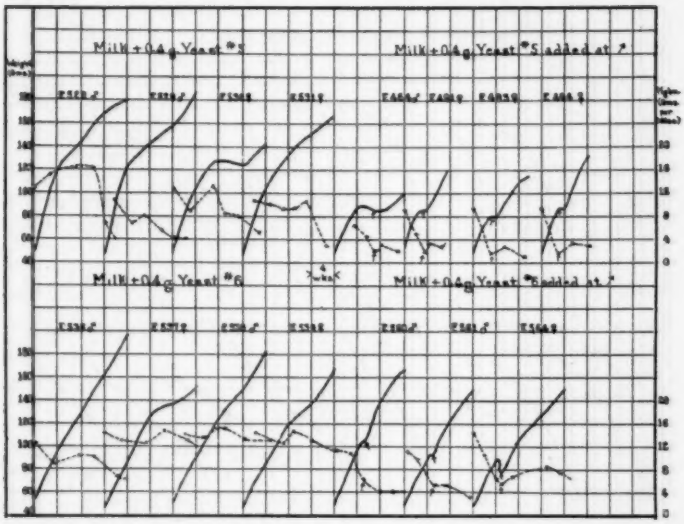

FIG. 4. Yeasts 5 and 6 came from the same company but were prepared in different ways. No. 5 contained less copper than any other sample and gave the poorest results. It was grown in a pure malt wort. No. 6 was grown in molasses wort, with ammonium salts.

## RESULTS

The antianemic potency of the various samples of yeast is shown graphically in figures 3 to 6. With 2 exceptions the yeasts showed some potency, the degree of effectiveness varying considerably. In general, they were more effective as prophylactics than as curatives. This was taken into consideration in giving

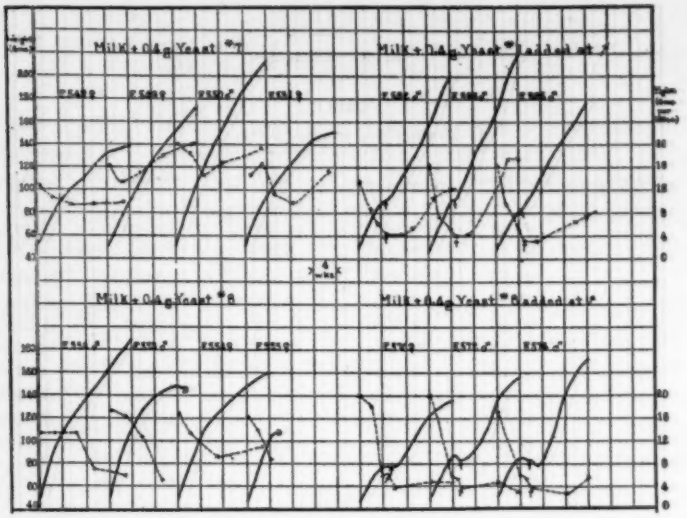

FIG. 5. Yeasts 7 and 8 came from the same company, but were prepared in different ways. No. 7 was grown on grain extract, while No. 8 was grown on molasses. No. 7 contained more copper than any other sample.

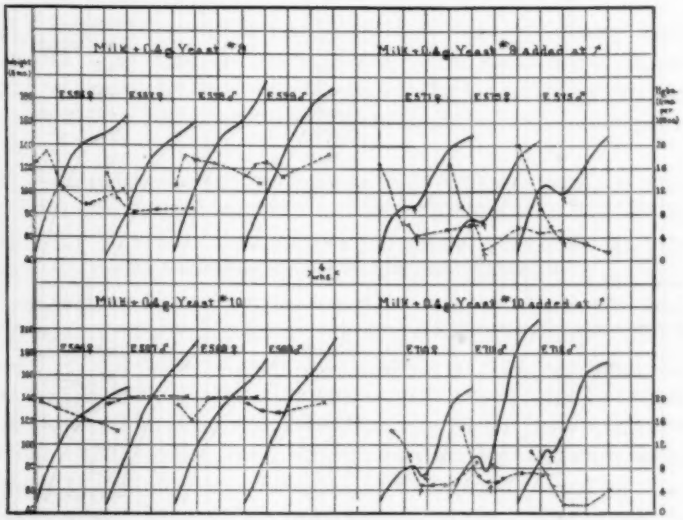

FIG. 6. Yeast 9 was low in both iron and copper. Yeast 10 was low in iron These samples were much more potent as prophylactics than as curatives.

TABLE 1

*Analysis of yeasts (per cent)*

| NUMBER | DRY MATTER | TOTAL NITROGEN | ASH | COPPER | IRON |
|---|---|---|---|---|---|
| 1 | 97.04 | 7.525 | 12.550 | 0.0032 | 0.0923 |
| 2 | 95.78 | 7.410 | 9.975 | 0.0035 | 0.0178 |
| 3 | 95.92 | 8.335 | 11.880 | 0.0041 | 0.0281 |
| 4 | 92.81 | 8.730 | 6.980 | 0.0045 | 0.0153 |
| 5 | 90.09 | 8.200 | 11.790 | 0.0022 | 0.0193 |
| 6 | 95.78 | 8.085 | 6.540 | 0.0038 | 0.0186 |
| 7 | 90.42 | 4.215 | 6.130 | 0.0056 | 0.0154 |
| 8 | 88.53 | 6.870 | 8.805 | 0.0034 | 0.0194 |
| 9 | 92.26 | 6.870 | 7.360 | 0.0026 | 0.0086 |
| 10 | 92.43 | 9.310 | 6.160 | 0.0046 | 0.0142 |

TABLE 2

*Relation between copper content and antianemic potency*

| NUMBER | PER CENT COPPER | ANTIANEMIC POTENCY |
|---|---|---|
| 7 | 0.0056 | Very good |
| 10 | 0.0046 | Good |
| 4 | 0.0045 | Good |
| 3 | 0.0041 | Very good |
| 6 | 0.0038 | Fair |
| 2 | 0.0035 | Poor |
| 8 | 0.0034 | Very poor |
| 1 | 0.0032 | Excellent |
| 9 | 0.0026 | Fair |
| 5 | 0.0022 | Very poor |

TABLE 3

*Relation between iron content and antianemic potency*

| NUMBER | PER CENT IRON | ANTIANEMIC POTENCY |
|---|---|---|
| 1 | 0.0923 | Excellent |
| 3 | 0.0281 | Very good |
| 8 | 0.0194 | Very poor |
| 5 | 0.0193 | Very poor |
| 6 | 0.0186 | Fair |
| 2 | 0.0178 | Poor |
| 7 | 0.0154 | Very good |
| 4 | 0.0153 | Good |
| 10 | 0.0142 | Good |
| 9 | 0.0086 | Fair |

the final rating to each sample. Yeasts 3 and 7 were particularly effective. Yeast 5 was practically without effect, while yeast 8 exhibited very weak hemoglobin regenerating power. Table 1 shows that yeasts 3 and 7 were rich in copper, that yeast 5 contained the least copper of any sample, while yeast 8 contained fair amounts of both copper and iron.

In order to see if any relationship could be shown to exist between the copper and iron content of the yeasts and their antianemic potency, the various samples are listed in table 2 in descending order according to their percentage copper content and classified according to their effectiveness as shown by the charts. In table 3 they are treated similarly according to their percentage iron content. With one notable exception the samples at the top of the list in table 2 are those that showed marked effectiveness. In table 3 no relationship between iron content and antianemic potency is apparent.

## DISCUSSION

The results obtained in this study show that yeasts vary considerably as to chemical composition. This may be due to the nature of the media, the vessels in which the yeasts are grown and the materials with which they come in contact during subsequent treatment.

It is likewise shown that yeasts from different sources vary considerably as to antianemic potency. In trying to link this up with their variation in chemical composition it becomes strongly indicative that it is rather closely related to their copper content. The superior value of yeasts 1 and 3 might be due to the high level of iron intake resulting from their administration. If the theory of the catalytic action of copper on iron is upheld this would seem to be a satisfactory explanation as it could be argued that the lower levels of iron intake had not reached the saturation point. On the other hand, yeast 7 furnished a relatively small amount of iron, but was richest of all the samples in copper, and showed strong hemoglobin regenerating power. This would indicate a direct utilization of the copper. However, in a complex substance such as yeast there are other inorganic substances

which were not determined and which might have exerted an influence.

Titus, Cave and Hughes (6) have obtained excellent results in hemoglobin regeneration through the use of manganese, while Myers and Beard (7) have obtained responses through the use of rarer elements, such as germanium, nickel, and cobalt. In view of the work of Waddell, Steenbock, Elvehjem and Hart (8), McHargue, Healy and Hill (9) and Krauss (10) the part played by copper, as shown in table 2, is outstanding.

Since the completion of the experimental work presented here, Sure and Kik (11) reported beneficial effects in hematopoietic function through the use of yeast. They found a greater hemoglobin concentration in nursing young when the mothers received a diet containing 10 per cent of dried yeast than when they received the stock diet. Sure, Kik, and Walker (12), referring to some of their own unpublished data, state that during gestation they find no anemia on synthetic diets containing an abundance of wheat embryo or yeast as sources of the vitamin B complex. In a later publication, Sure, Kik, and Walker (13) state that "the character of our results certainly does not show any relation between a deficiency of the vitamin B complex or uncomplicated vitamin B deficiency and pernicious anemia, or, as a matter of fact, does not establish the association with any definite form of anemia." It would seem, then, that the hemoglobin-regenerating power shown by certain samples of yeast is in no way related to their richness in the factors of the vitamin-B complex.

That the addition of yeast to an exclusive milk diet may exert some benefit other than in hemoglobin regeneration is indicated in the charts where it is shown that growth continues even after the hemoglobin has reached a low level. This may be due to greater food consumption and to an adequate intake of vitamin B. On an exclusive milk diet the growth curve follows the hemoglobin curve. Milk consumption decreases and, since milk has been shown to be a poor source of vitamin B (14), this may limit growth.

The iron requirements of infants on an exclusive milk diet are well known. If in the human, as in the rat, copper is a stimulus

for hemoglobin formation, some suitable substance furnishing both iron and copper should be administered. Hoobler (15) has fed yeast to infants with good results. This may have been beneficial not only because of the additional vitamin B (complex) furnished, but because of the increased intake of copper and iron.

## SUMMARY

Ten samples of yeast from various sources were assayed for antianemic potency. Seven samples showed definite hemoglobin regenerating power of varying degrees. Three samples showed very little or none.

Some correlation appeared to exist between the copper content of the yeasts and their antianemic potency. No such correlation was evident with respect to iron. Other substances, recently claimed to be concerned with hemoglobin regeneration, were not considered.

Some supplementary effect on growth was shown by the addition of yeast to milk. This was attributed to vitamin B.

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# LENGTH OF GESTATIONS IN JERSEY COWS*

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Duration of pregnancy is a problem ever confronting breeders of dairy cattle, for it is absolutely essential in the management of livestock for the owner or herdsman to have a definite knowledge as to when his pregnant cows are due to calve. The length of the gestation period is therefore of interest not only to the student of embryology but to every farmer who owns or cares for livestock. It is regrettable in view of the importance of the subject that the literature pertaining to the length of gestations is very meagre.

In recent years very few results of investigational work dealing with gestations in dairy cattle have appeared in print. Numerous gestation charts or tables have been published showing easy methods of figuring the "approximate" calving date. These tables do not all agree, some being based on gestations of 40 weeks, some on the basis of 282 days and others for shorter periods of time.

In 1817, M. Tessier (1) studied 575 cases and reported a variation from 241 to 321 days. The average was 282.2 days and 95 per cent of the calves were carried from 270 to 299 days. In a more extensive tabulation made by the Earl of Spencer (2) in 1840, the period varied between 220 and 313 days for calves born alive. The average was 283.3 days for 764 cases, including 23 twin births which averaged 277.2 days. About two-thirds (67 per cent) were from 280 to 289 days, 90 per cent were from 275 to 294 days and 95 per cent from 270 to 299 days. None born under 242 days were raised.

C. B. Bement (3), in 1845 found the variation to range between 276 and 299 days except for one short period of 213 days and a long one for 336 days. The average was 286 days (288 for 36 bull calves, and 283 for 26 heifers). Various breeds, Shorthorns,

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Devons, Herefords, Ayrshires, and grades were included. L. F. Allen (4) in 1868, reported a study of 50 cows of different breeds showing an average gestation of 284 days, with a range of 268 to 291 days.

H. H. Wing (5) tabulated 182, cases, mostly Holsteins and Jerseys. The range in the length of the gestation periods was from 264 to 296 days. The average found was lower than reported by the Earl of Spencer or Tessier being 280 days for 97 Holsteins and 279 days for 56 Jerseys. Five twin births averaged 275 days. Fitch, McGilliard and Drumm (6) in a study including four dairy breeds reported that the breeds varied in length of gestation as follows:

| | days |
|---|---|
| Jersey —100 gestations | 284.3 |
| Guernsey—103 gestations | 283.0 |
| Ayrshire —113 gestations | 284.6 |
| Holstein —220 gestations | 281.0 |

The average length of all the gestations studied was 282.4 days. The bull calves were carried 283.2 days and the heifers 281.9 days.

## INVESTIGATIONAL

When a Register of Merit record is completed a calving affidavit is sent to the owner. It is retained by him and if the cow drops a living calf after finishing her test period, he fills in both the service and calving dates. The accuracy of these dates and the fact that the calf was alive at birth must be attested before a notary and then the affidavit is returned to the Breed Association office. If the cow does not calve within a certain length of time or if the calf is not alive at birth no affidavit is necessary.

This study concerns the records accepted by the American Jersey Cattle Club during 1927 for which calving affidavits were returned swearing that living calves were dropped. It included 1075 gestations, all of which were apparently normal, if a living calf can be assumed as evidence of normality. The length of each of these gestation periods was determined and also the sex of the calf in all cases where the progeny had been registered. In

computing the length of the gestation period the day on which the calf was born was included in the total number of days, but not the day of service. For example, if a cow was served on January 12 and calved on October 18 the length of the period of pregnancy would be considered as 279 days. These gestations were then grouped according to the age of the dam at the time of calving. In table 1, are presented the data covering this phase of the investigation.

The shortest gestation reported was for 228 days and the longest for 312 days. Approximately 62 per cent of all the periods

TABLE 1

*Effect of age in length of gestation periods*

| AGE | NUMBER OF CASES | AVERAGE LENGTH OF GESTATIONS |
|---|---|---|
| Yearlings and Jr. 2 | 141 | 278.51 |
| Sr. 2 | 46 | 277.96 |
| Jr. 3 | 195 | 278.34 |
| Sr. 3 | 124 | 278.63 |
| Jr. 4 | 111 | 278.38 |
| Sr. 4 | 100 | 278.80 |
| 5 | 142 | 278.73 |
| 6 | 89 | 277.45 |
| 7 | 58 | 279.26 |
| 8 | 27 | 277.56 |
| 9 | 17 | 280.35 |
| 10 | 12 | 280.58 |
| 11 | 6 | 277.67 |
| 12 years and over | 7 | 281.43 |
| Total | 1,075 | 278.51 |

fall between limits of 275 and 283 days and 81 per cent come between 271 and 285 days. The average length of all the gestation periods was 278.51 days. However, only 68 of the 1075 calves were born on the 278th day of pregnancy, and the data indicate that there is almost an equal chance of the calf being born on any day after 271 days have elapsed and until the 285th day is reached. Table 2 illustrates how these gestations group themselves when arranged according to their length.

At the time this study was made, only 683 of the progeny had been registered in the Herd Books of the American Jersey Cattle Club. Of this number 297 were bull calves, 382 heifers and 4 sets of twins. On the basis of sex, the gestation periods of the 297 bull calves averaged 279.48 days and the 382 heifers averaged 278.64 days. The 4 sets of twins showed an average gestation of 275.50 days.

It was also of interest to find that while 63 per cent of the 1075 calves were registered, that of the 26 carried for 265 days or less only 11 or 42 per cent were registered while the 44 calves carried

TABLE 2

*Frequency table grouping gestations according to length*

| CLASS | FREQUENCY |
|---|---|
| 250 days and below | 5 |
| 251 to 255 | 4 |
| 256 to 260 | 10 |
| 261 to 265 | 7 |
| 266 to 270 | 46 |
| 271 to 275 | 206 |
| 276 to 280 | 384 |
| 281 to 285 | 325 |
| 286 to 290 | 61 |
| 291 to 295 | 19 |
| 296 to 300 | 6 |
| Over 300 days | 2 |

288 days or longer, 30 or 68 per cent were registered. Of the 5 calves carried for less than 250 days only one was raised, and of the 8 carried for 296 days or more, 5 were raised. This would indicate that those calves resulting from abnormally long gestations have a better chance of living than do those born prematurely.

Another question is whether certain cows do not possibly have a habit of carrying their calves either for shorter or longer period of time than the average for the breed. That is, if a heifer drops her first calf at 270 days, should the owner expect her following gestations to be shorter than usual. Twelve of those cows showing gestation periods longer than 290 days had at least 2 additional

recorded progeny and the same was true of 13 of the cows with gestations of 265 days or less. The life time breeding records of

TABLE 3

Progeny records of twelve cows carrying at least one calf for an abnormally long gestation period

| NAME OF COW | LENGTH OF GESTATIONS | SEX OF CALF |
|---|---|---|
| | days | |
| Flying Chief's Golden Beauty 480952 | 275 | F. |
| | 281 | M. |
| | 276 | M. |
| | 280 | F. |
| | 282 | M. |
| | 291 | M. |
| Tom's Lady Capitan 345664 | 280 | M. |
| | 280 | F. |
| | 279 | M. |
| | 280 | F. |
| | 280 | M. |
| | 280 | F. |
| | 299 | * |
| | 281 | M. |
| | 278 | M. |
| Able Fox's Rainbow 566179 | 273 | F. |
| | 275 | F. |
| | 280 | M. |
| | 293 | M. |
| Miss Dumont's May 566269 | 280 | F. |
| | 279 | F. |
| | 280 | F. |
| | 289 | M. |
| | 310 | M. |
| Fomasa's Irene 550669 | 282 | F. |
| | 282 | F. |
| | 290 | * |
| Queen Ladette of Bryan 628569 | 281 | F. |
| | 284 | M. |
| | 295 | M. |
| Oxford's Royal Nora 595773 | 279 | M. |
| | 287 | M. |
| | 286 | M. |
| | 290 | F. |
| Design's Dairylike Ildg 663202 | 273 | M. |
| | 276 | F. |
| | 299 | F. |
| Campanile's Pansy 585974 | 280 | F. |
| | 281 | F. |
| | 285 | F. |
| | 291 | F. |
| Belle's Huckleberry 521808 | 279 | M. |
| | 274 | F. |
| | 296 | * |
| Knight's Dolly Dimple 510442 | 289 | F. |
| | 282 | F. |
| | 282 | M. |
| | 285 | M. |
| | 297 | F. |
| Sir Owl's Jewel's Julia 640298 | 272 | F. |
| | 292 | F. |
| | 279 | M. |

* Not registered.

TABLE 3—*Continued*

| NAME OF COW | LENGTH OF GESTATION | SEX OF CALF | NAME OF COW | LENGTH OF GESTATION | SEX OF CALF |
|---|---|---|---|---|---|
| **Progeny records of thirteen cows carrying at least one calf for an abnormally short gestation period** | | | | | |
| | days | | | days | |
| Passport's Jap's Princess 616755 | 281 | F. | Jap's Poetess 460418 | 283 | F. |
| | 266 | M. | | 281 | F. |
| | 261 | F. | | 290 | F. |
| | | | | 237 | * |
| The Kid of Ohio's Gusta 654807 | 279 | F. | Prince's Louise M 494446 | 279 | M. |
| | 259 | * | | 290 | M. |
| | 278 | M. | | 231 | * |
| Majesty's Hildegarde 558997 | 279 | F. | Golden Fontaine You'll Do 664077 | 280 | F. |
| | 278 | F. | | 280 | M. |
| | 228 | * | | 265 | * |
| Sovictor Lenore 543955 | 279 | M. | Imp. Beaulieu Comtesse 692510 | 283 | M. |
| | 285 | M. | | 275 | M. |
| | 264 | F. | | 251 | M. |
| Dolly's Milkmaid 575423 | 279 | M. | Tiddledywink's Princess Eva 645558 | 282 | F. |
| | 284 | F. | | 256 | * |
| | 265 | M. | | 282 | M. |
| Star's Jolly T. A. 598467 | 282 | M. | Owl Knight's Lady 526650 | 280 | F. |
| | 278 | F. | | 280 | F. |
| | 282 | F. | | 281 | F. |
| | 281 | F. | | 281 | M. |
| | 256 | F. | | 239 | * |
| Jewel's Majestic Betsy 588297 | 273 | F. | | 279 | F. |
| | 285 | F. | | | |
| | 257 | F. | | | |
| **Jersey one-thousand-pound fat producers with four or more registered progeny** | | | | | |
| Abigail of Hillside 457241 | 277 | F. | California's Rinda's Insie 565559 | 280 | F. |
| | 284 | M. | | 271 | F. |
| | 282 | M. | | 278 | F. |
| | 277 | M. | | 276 | M. |

* Not registered.

TABLE 3—*Continued*

| NAME OF COW | LENGTH OF GESTATIONS | SEX OF CALF | NAME OF COW | LENGTH OF GESTATIONS | SEX OF CALF |
|---|---|---|---|---|---|
| Jersey one-thousand-pound fat producers with four or more registered progeny—*Continued* | | | | | |
| | *days* | | | *days* | |
| | 285 | M. | | 292 | M. |
| | 277 | M. | | 266 | M. |
| | 277 | F. | | 275 | F. |
| Darling's Jolly Lassie 435948 | 281 | F. | Lad's Iota 350672 | 277 | F. |
| | 286 | M. | | 272 | F. |
| | 280 | F. | | 254 | F. |
| | 278 | F. | | 274 | M. |
| | 281 | M. | | | |
| | 276 | M. | | 278 | F. |
| | 281 | F. | | 280 | M. |
| Prince's Emma of H. S. F. 359390 | 284 | F. | Madeline of Hillside 389336 | 278 | M. |
| | 277 | F. | | 284 | M. |
| | 274 | F. | | 285 | M. |
| | 275 | F. | | 282 | F. |
| | 286 | M. | | 287 | F. |
| Lady's Silken Glow 313311 | 278 | F. | | 284 | F. |
| | 281 | F. | Plain Mary 268206 | 288 | M. |
| | 284 | M. | | 268 | M. |
| | | | | 288 | M. |
| | 252 | F. | | 274 | F. |
| | 276 | M. | | | |
| | 278 | M. | | 275 | M. |
| St. Mawes Lad's Lady 451568 | 283 | M. | | 265 | F. |
| | 272 | F. | | 277 | M. |
| | 278 | F. | Vive La France 319616 | 276 | M. |
| | 279 | M. | | 271 | F. |
| | | | | 286 | M. |
| | 281 | M. | | 284 | M. |
| | 276 | F. | | | |
| | 279 | M. | | | |
| | 282 | F. | | 273 | F. |
| Red Lady 396118 | 275 | M. | | 285 | M. |
| | 282 | F. | Fauvic's Star 313018 | 280 | F. |
| | 282 | F. | | 273 | F. |
| | 284 | M. | | 259 | M. |

TABLE 3—*Concluded*

| NAME OF COW | LENGTH OF GESTATIONS | SEX OF CALF | NAME OF COW | LENGTH OF GESTATIONS | SEX OF CALF |
|---|---|---|---|---|---|
| Jersey one-thousand-pound fat producers with four or more registered progeny—*Concluded* | | | | | |
| | *days* | | | *days* | |
| Princess Elise 418113 | 271 | F. | Princess Xenia 356699 | 282 | F. |
| | 278 | M. | | 280 | F. |
| | 276 | F. | | 278 | F. |
| | 285 | F. | | 281 | M. |
| | 285 | F. | | | |
| St. Mawes Lad's Pride 515044 | 273 | F. | Tiddledywink's Quality Girl 560784 | 279 | F. |
| | 267 | F. | | 279 | F. |
| | 272 | M. | | 278 | M. |
| | 295 | M. | | 277 | M. |

these 25 cows were studied. Also, investigation showed that 17 of the 24 Jersey cows with butterfat records over one thousand pounds of fat were the dams of 4 or more registered calves. It was believed that the breeding records of such cows would be the most reliable obtainable and the gestation periods of their progeny are therefore given in table 3.

Examination of the breeding records of the cows in these groups does not reveal any correlation between the length of pregnancies for the same individual dam. In fact a great deal of variation is noted in most cases. In the first group the 12 extremely long gestations averaged 295.25 days in length and the other 40 gestations of these same cows averaged 280.15 days. In the second group the 13 abnormally short gestations averaged only 251.46 days in length while 32 other gestations by these 13 dams averaged 280.47 days. From these records it does not seem possible to predict or determine the probable length of a future gestation period even though the length of several previous periods are known. Included in the 3 preceding groups are 186 gestations. The average length of all of these gestations is 278.19 days. However, since the accuracy of the service dates of these preg-

nancies have not been attested to they were not included in the 1075 gestations on which the main portion of this study was based.

## SUMMARY

1. The average gestation period for the 1075 cases studied was 278.51 days but there appears to be an equal chance of a cow calving anytime between the 271st and the 285th day.

2. The age of the dam apparently has no effect on the length of gestation.

3. Bull calves were carried an average of one day longer than heifer calves, 297 bull calves being carried for an average of 279.48 days and the gestations of 282 heifer calves averaging 278.64 days.

4. The data are too scant to draw conclusions relative to twin births but the results compare with those obtained by other investigators in that the gestations of twins are usually several days shorter than the average.

5. Calves resulting from abnormally long gestations seem to have a better chance of living than do those born prematurely.

6. No correlation could be detected between the length of different gestations in the same individual dam. Cows showing one or more short gestations also exhibited other gestations longer than the average and vice versa.

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# QUANTITATIVE CHANGES IN THE MICROFLORA OF BUTTER DURING STORAGE*

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In connection with studies concerning factors which influence the market quality of butter, data have been accumulated on the mold, yeast and bacterial counts of butter kept in storage for various periods of time and at different temperatures. These data are presented in the belief that they may be useful in the interpretation of changes occurring in the microflora of butter.

Several lots of butter were available for study. Lot 1 represented 63 samples of salted butter manufactured in 8 different creameries in Minnesota during August, 1927. This butter was in 1 pound prints wrapped and stored at the creamery for 1 to 6 days at 30° to 45°F. and in transit at 42° to 67°F. for 7 to 8 days to the eastern markets through regular channels. Lot 2 consisted of 55 samples of salted butter manufactured at the Minnesota State Experimental Creamery during the period of one year (1926–1927), packed in 3 to 5 pound stone jars and kept for 1 month at 32° to 35°F. Lot 3 was made up of 68 additional samples of salted butter from the same creamery, packed in jars and stored at 32° to 35°F. for 9 months. Lot 4 consisted of 297 samples of salted butter, mostly in 20 pound tubs, manufactured in various creameries throughout the country and entered in the 1927 storage contest of the National Creamery Buttermakers' Association. The butter was stored at −5 to −10°F. for a period of 5 months. Lot 5 represented 55 samples of unsalted butter from the same churnings as lot 2, stored in the same type of package, and for the same period. Lot 6 was made up of 68 samples of unsalted butter from the same churnings as lot 3 and kept under the same conditions. Thus, 483 samples of salted and 123 samples of unsalted butter were available for study.

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### METHODS

Mold, yeast and bacterial counts were made for all samples before and after storage. In the case of lots 1, 2, 3, 5, and 6 counts were made immediately after the butter was made while in the case of lot 4, the analyses were made at the time the butter went into storage, which meant that the butter was from a few days to a few weeks old before samples were available. All counts of storage butter were made when the butter was taken from storage or when it reached the market as in the case of lot 1. Samples were taken and platings made in the usual manner. Whey agar served as the medium, and in the case of mold and yeast counts, the agar was acidified with a tartaric acid solution previous to plating. Mold and yeast counts were made after incubation at 20° to 25°F. for 5 days unless plates were crowded in which case, counts were made at the end of 2 or 3 days. Bacterial counts were made after the plates had been incubated at 20° to 25°C. for 5 days and an additional period of 2 days at 37°C. All counts are expressed in terms of the number of colonies per cubic centimeter of sample.

### PRESENTATION OF DATA

When the data were assembled it was found that the results obtained with lots 1, 2, 3 and 4 were so generally consistent that it was not considered necessary to include, in this report, the results from each individual lot of butter but to present the combined results representing all samples of salted butter. The same situation was true of the results from the two lots of unsalted butter, consequently the figures given represent all unsalted samples. The differences in the results obtained under different temperatures and periods of storage were not pronounced and did not influence the general trend.

The data presented in table 1 show the range in mold, yeast and bacterial counts for all samples, before and after storage. It will be noted that the majority of mold counts of fresh butter, salted or unsalted, are below 10 per cubic centimeter. After storage a greater proportion of the salted butter had such a low count

while the proportion of low counts in the unsalted butter had diminished, so that the major portion of counts were in the hun-

TABLE 1

*Distribution of counts in fresh and stored, salted and unsalted butter*

| COUNTS | DISTRIBUTION OF COUNTS | | | |
|---|---|---|---|---|
| | Before storage | | After storage | |
| | Salted | Unsalted | Salted | Unsalted |
| *Molds per cubic centimeter* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0–10 | 73.5 | 61.8 | 88.0 | 22.8 |
| 11–50 | 18.6 | 24.4 | 7.3 | 10.6 |
| 51–100 | 2.1 | 5.7 | 0.8 | 2.4 |
| 101–500 | 2.9 | 3.3 | 1.6 | 14.6 |
| 501–2,500 | 1.2 | 3.3 | 2.1 | 10.6 |
| 2,510–10,000 | 1.5 | 1.5 | 0.0 | 15.4 |
| 10,100–50,000 | 0.2 | 0.0 | 0.0 | 11.4 |
| 51,000–250,000 | 0.0 | 0.0 | 0.2 | 4.9 |
| 251,000–1,000,000 | 0.0 | 0.0 | 0.0 | 6.5 |
| 1,100,000–5,000,000 | 0.0 | 0.0 | 0.0 | 0.8 |
| *Yeasts per cubic centimeter* | | | | |
| 0–10 | 36.4 | 53.8 | 47.4 | 25.2 |
| 11–50 | 25.7 | 23.5 | 21.3 | 11.8 |
| 51–100 | 7.9 | 2.5 | 6.6 | 14.3 |
| 101–500 | 14.7 | 12.6 | 10.1 | 16.8 |
| 501–2,500 | 10.6 | 5.9 | 10.1 | 21.0 |
| 2,510–10,000 | 2.9 | 1.7 | 3.5 | 6.7 |
| 10,100–50,000 | 1.4 | 0.0 | 0.4 | 3.4 |
| 51,000–250,000 | 0.4 | 0.0 | 0.3 | 0.8 |
| 251,000–1,000,000 | 0.0 | 0.0 | 0.3 | 0.0 |
| *Bacteria per cubic centimeter* | | | | |
| 0–1,000 | 9.7 | 17.2 | 23.4 | 1.5 |
| 1,010–10,000 | 27.1 | 27.0 | 32.3 | 5.7 |
| 10,100–50,000 | 25.3 | 13.1 | 25.7 | 10.7 |
| 51,000–250,000 | 21.1 | 9.8 | 9.9 | 19.6 |
| 251,000–1,000,000 | 9.9 | 13.1 | 6.0 | 26.2 |
| 1,100,000–10,000,000 | 6.9 | 15.6 | 2.7 | 27.9 |
| 11,000,000–66,000,000 | 0.0 | 4.2 | 0.0 | 8.4 |

dreds or thousands. This clearly shows the influence of salt upon the molds.

In considering the yeast count, one finds that the majority of

counts of the salted butter are below 50 per cubic centimeter. It is quite to be expected that the general level of yeast counts will be higher than the mold counts as previous data have consistently indicated. It is evident that the differences between the counts of fresh and stored butters are not as marked as they were in the case of the mold counts. The effect of salt is manifest but not as decided as it is for molds It has been generally found that yeasts are not as sensitive to higher salt concentrations.

The bacterial counts of the fresh butter are largely below 50,000 although a considerable proportion of the fresh unsalted butter had bacterial counts in the millions per cubic centimeter. The

TABLE 2

*Change in counts during storage of butter*

| | KIND OF BUTTER | TOTAL SAMPLES | DISTRIBUTION OF SAMPLES SHOWING | | |
|---|---|---|---|---|---|
| | | | Increase | No change | Decrease |
| | | | *per cent* | *per cent* | *per cent* |
| Molds | Salted | 483 | 22.6 | 12.6 | 64.8 |
| | Unsalted | 123 | 85.4 | 4.0 | 10.6 |
| Yeasts | Salted | 483 | 32.3 | 5.4 | 62.3 |
| | Unsalted | 119 | 71.4 | 3.4 | 25.2 |
| Bacteria | Salted | 483 | 25.3 | 1.0 | 73.7 |
| | Unsalted | 122 | 72.1 | 0.0 | 27.9 |

effect of salt is very marked in the case of the bacteria according to the data presented. A markedly increased proportion of the bacterial counts of salted butter are below 50,000 per cubic centimeter at the end of the storage period while the counts of the unsalted samples after storage are largely in the realm of millions. The influence of salt is much more marked than it was for yeasts or molds.

To approach the situation from another angle, consider the data presented in table 2 which show the relative increases or decreases in count during storage. The outstanding feature of these data is the marked differences between the changes taking

place in the salted and unsalted butter. The fact that 70 to 85 per cent of the unsalted samples increase in count during storage, while 60 to 75 per cent of the salted samples decrease is altogether in accordance with expectation and is in line with data previously available in the literature.

TABLE 3

*Ratio of increase or decrease in count during storage of butter*

(Ratio of decrease—Original count : final count :: $x$ : 1)
(Ratio of increase—Original count : final count :: 1 : $y$)

| RATIO | DISTRIBUTION OF SAMPLES ACCORDING TO RATIO OF CHANGE IN | | | | | |
|---|---|---|---|---|---|---|
| | Mold counts | | Yeast counts | | Bacterial counts | |
| | Salted butter | Unsalted butter | Salted butter | Unsalted butter | Salted butter | Unsalted butter |
| *Ratio of decrease (x:1) (x = original count)* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 100,001–250,000:1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| 25,001–100,000:1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 |
| 5,001–25,000:1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 |
| 1,001–5,000:1 | 0.2 | 0.0 | 0.4 | 1.6 | 0.2 | 0.7 |
| 251–1,000:1 | 0.9 | 0.0 | 0.6 | 0.0 | 1.7 | 1.6 |
| 51–250:1 | 1.1 | 0.9 | 3.5 | 2.5 | 7.7 | 4.1 |
| 11–50:1 | 9.9 | 2.4 | 10.8 | 8.4 | 20.0 | 7.4 |
| 1–10:1 | 52.8 | 7.3 | 47.0 | 12.6 | 43.0 | 13.9 |
| 0 | 12.8 | 4.1 | 5.4 | 3.4 | 1.0 | 0.0 |
| *Ratio of increase (1:y) (y = final count)* | | | | | | |
| 1:1–10 | 19.9 | 22.0 | 25.1 | 24.4 | 22.5 | 18.0 |
| 1:11–50 | 1.4 | 15.5 | 5.2 | 16.0 | 1.9 | 14.8 |
| 1:51–250 | 0.6 | 13.0 | 1.0 | 17.7 | 0.6 | 14.0 |
| 1:251–1,000 | 0.2 | 11.4 | 1.0 | 8.4 | 0.2 | 9.9 |
| 1:1,001–5,000 | 0.0 | 8.9 | 0.0 | 4.2 | 0.2 | 9.1 |
| 1:5,001–25,000 | 0.0 | 8.1 | 0.0 | 0.8 | 0.0 | 3.3 |
| 1:25,001–100,000 | 0.2 | 3.2 | 0.0 | 0.0 | 0.0 | 1.6 |
| 1:100,001–500,000 | 0.0 | 3.2 | 0.0 | 0.0 | 0.0 | 1.6 |

These figures do not demonstrate, however, the extent of increase or decrease which occurred in the various samples. Table 3 sets forth the results of calculations of the ratio between the original and final counts. In the determination of the ratio of decrease, the final count was taken as a basis, and as designated

in the upper portion of the first column as 1. In other words if the original count of a sample were 10,000 and the final count were 1000 then the ratio of *decrease* would be 10:1. The opposite calculation was made for the ratio of increase, so that if 1,000 were the original and 10,000 the final count, the ratio of *increase* would be 1:10. This method was considered as giving figures of some significance in showing the extent of increase or decrease in counts. As might be expected the largest percentage of the samples showed increases or decreases within the range of 1:10 or 10:1 respectively. In the case of the unsalted butter the greater tendency was toward larger increases as would be anticipated, especially of mold and bacterial counts. A study of

TABLE 4

*Effect of salt content on change in count during storage of butter*

| SALT CONTENT | TOTAL SAMPLES | DISTRIBUTION OF SAMPLES ACCORDING TO CHANGE IN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mold count | | | Yeast count | | | Bacterial count | | |
| | | Increase | No change | Decrease | Increase | No change | Decrease | Increase | No change | Decrease |
| *per cent* | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0.0 | 62 | 85.4 | 4.0 | 10.6 | 71.4 | 3.4 | 25.2 | 72.1 | 0.0 | 27.9 |
| 0.4–0.95 | 123 | 21.0 | 16.1 | 62.9 | 38.7 | 1.6 | 59.7 | 17.7 | 0.0 | 82.3 |
| 1.0–1.9 | 207 | 15.9 | 13.6 | 70.5 | 33.3 | 4.9 | 61.8 | 27.5 | 0.5 | 72.0 |
| 2.0–3.4 | 213 | 29.0 | 10.7 | 60.3 | 29.1 | 6.6 | 64.3 | 26.3 | 1.9 | 71.8 |

the data did not indicate that there was any consistent relationship between low counts and a high ratio of increase or high counts and high ratio of decrease. The increase or decrease apparently was more a function of the types of organisms present in the sample rather than the numbers of them.

Inasmuch as there exists such a marked difference between the changes in salted and unsalted butter, a comparison was made between the changes in samples of different salt content. Table 4 gives the results of such a comparison. It will be noted that there is a sharp line of demarkation between the unsalted and salted samples even when less than 1.0 per cent of salt was present. It is interesting to observe that there was no greater tendency for the more highly salted samples to decrease in count than for those of

lower salt content. This may be explained by the fact that the majority of organisms checked by salt are inhibited by the presence of ralatively small amounts of it. This is especially true in the case of the molds where *Oospora lactis* is the principal type encountered on plates poured from butter and one of the types most susceptible to salt concentration. The data were also studied from the standpoint of the concentration of salt in the brine but the results were practically identical with those presented in table 4. There was a slight indication that the more concentrated brines were somewhat more inhibiting but not strikingly so.

The data in general are interesting and suggestive. The tendency for microörganisms in butter to be checked in their growth by the presence of salt has often been noted. The development of microörganisms in unsalted butter is more extensive as might be expected. One does not interpret qualitative changes in butter on the basis of quantitative changes in the flora but the fact that microörganisms are able to show development in some samples of salted butter and more generally in unsalted butter is suggestive that one cannot overlook the quantitative changes entirely. If some organisms can grow, even though they are harmless, and merely constitute the major portion quantitatively, the more noxious types may also be developing to the detriment of the quality of the butter.

## SUMMARY

1. Mold, yeast and bacterial counts of 483 samples of commercial salted butter and 123 samples of unsalted butter, before and after storage, are reported.

2. There is a general tendency for salted samples to show decreases in counts during storage.

3. There is an equally significant tendency for unsalted samples to show an increase in count.

4. The ratio of increase is higher in the unsalted than in the salted butter while the opposite is true for the ratio of decrease.

5. The salt content of butter has a decided effect upon quantitative changes in the microflora of butter during storage but this is not in proportion to the amount of salt present.

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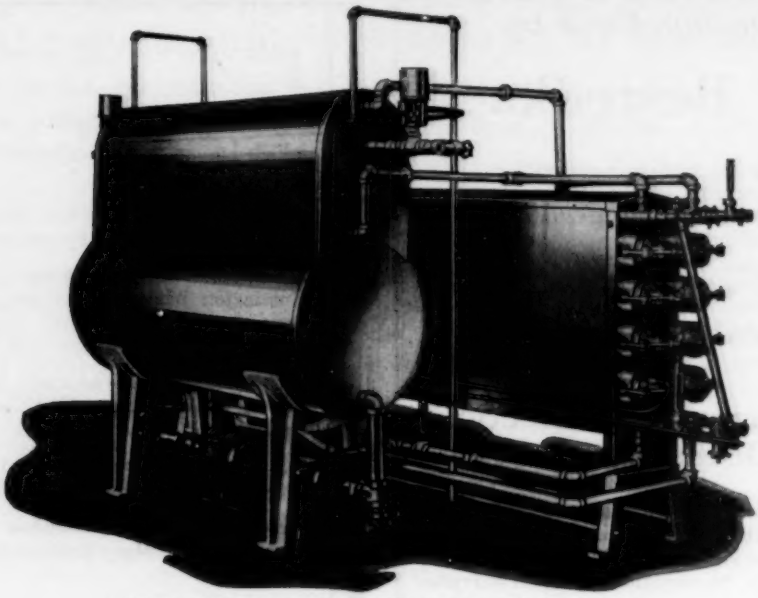

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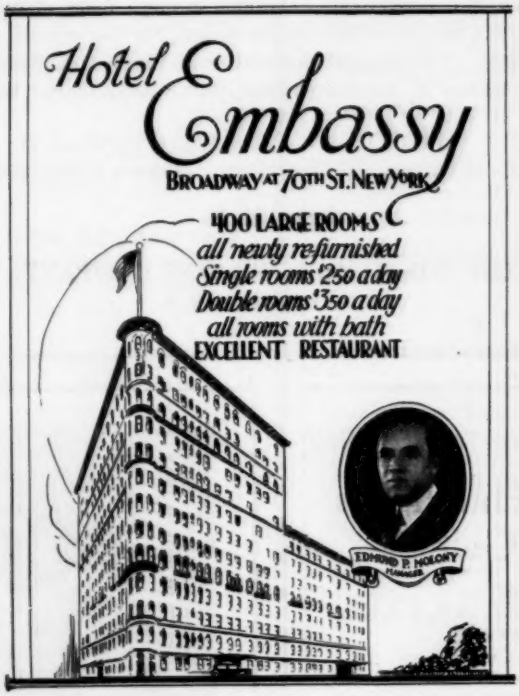


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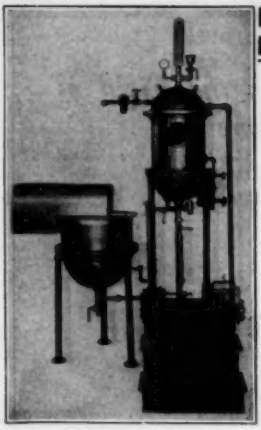

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